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COOPERATIVE AGREEMENT NUMBER DAMD 17-97-2-7013

TITLE: MILITARY NUTRITION RESEARCH: EIGHT TASKS TO ADDRESS MEDICAL FACTORS LIMITING SOLDIER EFFECTIVENESS

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REPORT DATE: July 1998

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 1998	Annual (1 Jul 97 - 30 Jun 98)

4. TITLE AND SUBTITLE
Military Nutrition Research: Eight Tasks to Address Medical Factors Limiting Soldier Effectiveness

5. FUNDING NUMBERS
DAMD 17-97-2-7013

6. AUTHOR(S)
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*
To assess, maintain, or improve a soldier's physical/physiological/psychological capability to function effectively under environmental and operational stress and to minimize adverse effects of stress on health safety and performance, the PBRC will perform the following eight research tasks: 1) Clinical Laboratory for Human and Food Samples will perform laboratory analysis of samples from studies conducted by the U.S. Army Research Institute of Environmental Medicine (USARIEM) and at PBRC in Tasks 4 and 8. 2) Stable Isotope Laboratory will perform analyses to measure the energy expenditure and body composition of soldiers during prolonged field exercise and at PBRC in Tasks 4 and 8. 3) Stress, Nutrition and Mental Performance Laboratory will continue multidisciplinary basic research studies of the interactions of stressors and nutrition on mental performance parameters in an animal model. This lab collaborates with Task 7 where samples are sent to evaluate immune function in the stressed animal model. 4) Stress Nutrition and Work Performance uses human subjects to develop nutritional strategies to improve physical performance under intense physical stress and to collaborate with Task 7, sending samples for analysis to link muscular fatigue to decrements in immune function. 5) Nutrient Database Integration Laboratory supports the Military Nutrition Division and PBRC research studies by providing dietary intake and analysis support. 6) Enhancing Military Diets continues to expand the Armed Forces Recipe file with healthier recipes. 7) Stress Nutrition and Immune Function Laboratory provides special tests of immune function in collaboration with Tasks 3 and 4 to evaluate immunologic change in humans and in a rat model. 8) Metabolic Unit Project allows new inpatient protocols to address specific issues in nutritional interactions with stress which affect performance and immune function.

14. SUBJECT TERMS :	15. NUMBER OF PAGES
military nutrition research; stress, nutrition and mental performance; stress, nutrition and work performance; enhancing military diets; stress, nutrition and immune function	156
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

FOREWORD

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Donna H Lynn 6/22/98

PI - Signature Date

TABLE OF CONTENTS

ANNUAL REPORT
US ARMY GRANT #DAMD 17-97-2-7013

Introduction

On April 1, 1997, Grant #DAMD 17-97-2-7013 was awarded to Pennington Biomedical Research Center (PBRC) to address the following **hypothesis: Military Nutrition Research: Eight: Eight Tasks to Address Medical Factors Limiting Soldier Effectiveness.**

The goal of this research is to assess, maintain, or improve a soldier's physical/ physiological/psychological capability to function effectively under environmental and operational stress and to minimize adverse effects of stress on health, safety and performance.

Technical Objective

This research continues the research relationship between the PBRC and USARIEM over a five year period. Those research relationships were established under prior cooperative agreements, #DAMD 17-88-Z-8023, "The effect of food, diet and nutrition on military readiness and preparedness of military personnel and dependents in a peace time environment," and #DAMD 17-92-V-2009, "Military nutrition research: six tasks to address medical factors limiting soldier effectiveness."

The project allows for the continuation of the Clinical Laboratory for Human and Food Samples, Stable Isotope Laboratory, Menu Modification Project, and Nutritional Neuroscience Laboratory, all of which were initiated under Grant #DAMD 17-88-Z-8023. The project also expands the scope of research to allow for studies in humans of stress, nutrition and work performance, and for studies in humans and animals to evaluate the relationship of stress, nutrition and immune function. The grant provides a nutrient database laboratory. The project also allows for the utilization of the PBRC's inpatient metabolic unit for a study designed by USARIEM investigators as detailed in the Metabolic Unit Project section.

Military Significance and Relevance to USARIEM Needs

The Stable Isotope and Clinical Laboratory methodologies are critical components of in-house military nutrition research of the U.S. Army Research Institute of Environmental Medicine. These extramural projects provide critical capabilities that do not exist in house, but are needed to fulfill the Army Surgeon General's responsibility to provide nutritional research support to the DOD and Nutrition RDT&E Program.

The Clinical Laboratory also provides support for three projects of Defense Women's Nutritional Health Research.

The Nutritional Neuroscience Laboratory expands our knowledge of the effects of stress and sleep deprivation and explores the ameliorative effects and mechanisms of action of dietary-induced alterations in behavior and cognitive function. Advances in this knowledge are the basis for developing safe and effective nutritional strategies to sustain and enhance soldier performance under conditions of environmental or operational stress. The project also provides insight into the roles of corticotrophin releasing factor (CRF) and locus coeruleus (LC) noradrenergic mechanisms in mediating anxiety in rats exposed to restraint stress.

The Menu Modification Project fulfills military needs to promote health, maintain readiness and sustain soldier performance. The Nutrient Database Integration Laboratory supports USARIEM projects assessing food intake in the field.

The Metabolic Unit Project fulfills military need for an inpatient site for performance of specialized research utilizing the body composition assessment, energy expenditure assessment, metabolic kitchen services, and clinical laboratory expertise of the PBRC.

This annual report describes progress during the first year of the grant. Discussions of individual projects funded under this grant follow.

- In the third quarter of the first year of the grant, we submitted our annual use report to the Animal Care and Use Division. This can be found in the appendix. We also provided a fiscal year report (10/1/96-9/30/97). This is also attached in the appendix.

- On November 18-19, 1997 we were visited by Dr. Harris Lieberman and the Armed Forces Recipe Services and Armed Forces Food Policy Council. Dr. Lieberman reviewed the overall grant activities with these two organizations. The purpose of their visit to the Pennington Center was to determine capabilities of the Center to provide future support in recipe development and evaluations for armed forces recipes and to clarify procedures for submitting recipes currently being developed under the existing grant. The visitors expressed satisfaction with our current procedures and outlined guidelines for our continued support of their recipe development endeavors. They also expressed desire for our food preference survey to be administered at military installations of all services, not only the Army.

The six tasks performed under this project are listed and described below.

I. Clinical Laboratory for Human and Food Samples

A. Introduction

The Clinical Research Laboratory, which functions as a support laboratory for the U.S. Army's nutritional research program, continued to receive and analyze samples for the Army in 1997-1998. Although the number of studies decreased in number this year, the clinical and food analysis laboratories were still key to several studies. The laboratory continues to offer a broad

spectrum of analytical tests in support of army research. In addition to the standard tests performed in the past a few new methods were put on line and new instrumentation was obtained (see below).

B. Body

In the past year the laboratory replaced two accessioner/phlebotomists (Angel Rainwater, Tina Eccles) and added two more (Stephen Lacour and Fran Baker). In the Food Analysis Laboratory, Regina Louviere, one of our Research Associates, resigned and was replaced by Dianne Ratcliffe. Dianne was trained and is now performing analyses.

A new nitrogen analyzer was put out for bid and received. The instrument is now in operation in the clinical lab. A new Beckman Synchron CX5 was obtained on which to run user defined chemistries. This has been installed and is operational.

Several people participated in Army field studies and aided in the processing of samples for subsequent analyses. Three draws of the Sergeant Major's Academy study in El Paso were attended by a total of 9 people. Three people (Ronnie Bodin, Tina Eccles, and Richard Tulley) also helped in processing of samples.

Results for the majority of the chemistries ordered for the Sergeant Major's Academy study (first, second, and third draws) were sent in April, 1997 to Major Karge. Results are still pending for homocysteine, awaiting method development.

Work was completed on the development of a method of analysis of amino acids by HPLC with pre-column derivatization with OPA (see appendix). Analyses for Ranger 3 amino acids were completed. Dr. Lieberman was sent these results in May, 1997.

The analysis of Vitamins A and E were completed for the Sergeant Major's Academy Study and the SAFS-5 study.

A final shipment of samples from the Banderet Tyrosine study were received and all results were completed. A correlation study of a new catecholamine method by RIA proved disappointing, therefore catecholamines were analyzed by our old HPLC method for this study. Plasma catecholamines and prolactin analyses were completed for this study. These results were sent to Dr. Bandaret. Analyses for this study are now complete.

Data was cleaned up and analyzed statistically for the Sleep Deprivation Study.

C Reactive Proteins were also requested by Major Bill Karge on the Sergeant Major's Academy Study. These were run but showed very low values.

Additional analyses for the Sergeant Major's Academy Nutritional Survey study were completed. These included C Reactive Protein, vitamin B12, and folate.

Red cell enzyme assays for the CASH study and for the Hot Weather Feeding Study were completed.

Most of the tests for the CASH study were completed. The exceptions are the homocysteine assay and vitamin A, E, and carotenoids.

A method for the analysis of vitamin C by capillary electrophoresis was developed. Excellent speed and resolution were achieved. Sensitivity is approximately 4-5 mg/L, limiting this method for low values, however, the method could be used as a discriminator between normal and low nutriture. An attempt to improve sensitivity was made by using Hewlett Packard's new high sensitivity flow cell, however, results have not been completely satisfactory to this point. It is hoped that correlation studies will be conducted between the capillary electrophoresis method and the in-house developed automated method.

A method for the analysis of homocysteine by capillary electrophoresis was investigated. The first method tried was an indirect method using dimethyl aminobenzoate and alpha cyclodextran. This method was able to resolve homocysteine, however, appears to suffer from lack of sensitivity. We hope to continue to investigate methods for homocysteine by capillary electrophoresis.

Work was done on the development of a method of analysis of homocysteine by HPLC. This method showed great promise. Separation and resolution were very good. We were at the point of performing precision and recovery studies when the column had to be replaced. Results with this new column, however, were disappointing. Even though this column was identical in type, order number, etc., the column packing was of a different batch and did not exhibit the resolution of the first column. We are awaiting a replacement column from the company to continue our development.

A method for the analysis of 3-methyl histidine using our amino acid method of HPLC showed what appeared to be good separation and recovery but gave disappointing results. Results showed values which were higher than expected physiologically. We believe there are interfering substances present. New methods are being investigated.

A shipment of samples from two studies was received in January. Major Jeff Kennedy, no longer with the Army, sent samples from the Ranger 4 study and SAFS-6 study. He asked for us to run TSH, T3 free and total, T4 free and total, TBG, GH, IGF-1, liver function tests, cholesterol, HDL, triglycerides, vitamin A, vitamin E, folate, beta carotene, sodium, potassium, calcium, BUN, and glucose on both studies. We are still awaiting word from Major Karge on the status of these samples.

Another shipment from the Carbohydrate and Performance study by Steve Lewis was received. Analyses have been completed for this study and a preliminary report was sent to Major Lewis in March. A formal report will be forthcoming.

The new nitrogen analyzer was put in use and doing very well for urine nitrogens; however, it is still having problems with digested samples (fecal and food). The problem is apparently due to the catalyst or strong acid used in the digestion. Since this instrument uses a direct needle injection rather than a boat as in the old instrument, there are problems with the sample needle getting quickly blackened. For some reason very low counts are being obtained on these samples. We are still working with the company to solve the problem.

C. Conclusions

The clinical laboratory performed testing for five studies and worked on methods for four analytes and a catecholamine method evaluation was performed. Two field studies were attended by laboratory personnel to aid in the processing of samples.

D. References

None.

II. Stable Isotope Laboratory

A. Introduction

The research conducted by the Stable Isotope Laboratory is in the area of energy and water requirements, and changes in body water, of soldiers, often under harsh environmental conditions. The method used to determine energy requirements is the doubly labeled water (DLW) technique, which involves oral administration of water labeled with the stable isotopes, ^{2}H and ^{18}O. Saliva and urine samples are then obtained for periods of 4-14 days, longer with redosing. Water intake can be determined using only the ^{2}H labeled water.

The use of doubly labeled water for measurement of energy expenditure was developed as a field technique for use in small animals (1). The method is based on the premise that after a loading dose of $^{2}H_{2}{}^{18}O$, ^{18}O is eliminated as CO_2 and water, while deuterium is eliminated from the body as water. The rate of CO_2 production, and, hence, energy expenditure, is calculated from the difference of the two elimination rates. Doubly labeled water, using the two-point method, is an ideal method for use in free-living subjects because it is noninvasive and nonrestrictive. The only requirement of subjects is to give urine and saliva specimens before and after drinking an initial dose of $^{2}H_{2}{}^{18}O$, and then return in one to two weeks to give a final urine specimen. During the period between the two urine and saliva samplings, subjects are free to carry out their normal activities and are not required to maintain extensive diaries.

The doubly labeled water method has been extensively validated in humans under controlled settings (2), but there are confounding factors that need to be considered in field studies, particularly in Army Field Studies. Among these are change in location or food and water supply immediately preceding, or during an energy expenditure study. These changes may cause a change in baseline isotope abundance and, therefore, interfere with the accuracy of the energy expenditure measurement. This has occurred in a previous field training exercise involving the study of the MRE and RLW rations (3). This is a particular problem with studies such as the Ranger Training Studies (4), in which soldiers are moved to different parts of the country during the study. Therefore, a group not receiving labeled water must be followed to make any corrections in baseline isotope shifts.

Hydration status is another main focus for some Army studies. Using the cheaper and more readily available deuterium tracer, either changes in total body water (5,6) can be followed during a study, or water turnover (intake) (7,8) can be measured during a study.

One advantage of the DLW method is that it uses stable isotopes so there is no radiation exposure. The method uses two heavy isotopes of water, which are naturally occurring in food and water. There are no known side effects of either isotope at the doses given in DLW studies and has been used extensively to study energy expenditure during pregnancy (10,11) lactating women (12), and infants for measurement of energy expenditure and human milk intake (13-15).

The Stable Isotope Lab was involved in several Army research projects during the current year. These are described below.

B. Body

Stable isotope studies were completed for 3 studies and a fourth large study was begun. The first study completed was a DLW study for the 75th Ranger Regiment Study in Savannah, in which there were two phases, the second phase being a Field Training Exercise (FTX). The second study completed was a large, long term study in Rangers which included 4 separate DLW dosing periods. The 3rd study completed was a study in which Norwegian Rangers underwent an intense training exercise under harsh environmental conditions.

The isotope analyses for the 75th Regiment Study (Savannah) were completed this year. The deuterium and Oxygen-18 baseline isotope shifts for the placebo subjects (See 21st Quarterly Report) were calculated to correct the isotope data for the labeled subjects. The individual energy expenditure values for the two periods are given in a table 21st Quarterly Report. The raw data for TBW determinations and deuterium and O-18 elimination rates are presented in the Appendix (21st Quarterly Report). The mean energy expenditures in barracks and in the field were 4200±800 kcal/d and 4800±900 kcal/d.

The Ranger Study completed this year is different from our past Ranger studies in that we obtained energy expenditure data for the entire period, from 12 Feb, through 10 Apr, by doubly

labeled water (DLW). Four DLW doses were administered, 12 Feb, 25 Feb, 9 Mar and 24 Mar, to cover the entire period. The isotope analyses for the Ranger 96 study were completed, and final calculations been made. The raw data, as well as the summary sheet for energy expenditure calculations from repeat analyses are given in the Appendix (22nd Quarterly Report). Energy expenditure during each period was quite high, nearly 5500 kcal/d for periods 1-3, and just under 5000 kcal/d for the final period (See 22nd Quarterly Report).

Isotope analyses and calculations were completed for the Norway '97 study. Baseline isotope shifts (D_2O and $H_2{}^{18}O$) for the two subjects not receiving isotope (see 23rd Quarterly Report) were used to adjust isotope enrichments in the subjects receiving the $D_2{}^{18}O$. Energy expenditure was calculated by calculating elimination rates of deuterium and ^{18}O by linear regression, using the 6/3, 6/4, 6/9 and 6/10 time points. Since these soldiers received very little food, and hence relied largely on their fat stores for energy, we used an assumed RQ of 0.75 for conversion of CO_2 elimination to energy expenditure. There was also a discrepancy with the ^{18}O dose, leading to calculation of a larger total body water (TBW) calculated form ^{18}O compared to deuterium. That the higher TBW calculated from ^{18}O dilution was incorrect was confirmed by comparison of FFM estimated from calculated TBW from deuterium and ^{18}O dilution spaces, compared to that obtained by DEXA measurements. Therefore, we used the D_2O dilution spaces for calculation of energy expenditure, as given in the table below. The mean energy expenditure in this study was very high, 5650±800 kcal/d.

Discussions with Reed Hoyt and Bill Tharion at USARIEM were conducted for the execution of the current study employing doubly labeled "Effects Of Tray Ration Consumption During A 63-Day Marine Field Exercise." Doubly labeled water measurements of total daily energy expenditure and water turnover will be obtained during 3 periods throughout the 63 day study. The three periods for which data will be captured are:

1 Dose on 31 March, April 1 through April 9
2 Dose on May 2, May 3 through May 7
3 Dose on May 23, May 24 through May 27

Samples from the field have been received and logged in the stable isotope lab. Sample cleanup has begun. Isotope analyses will begin shortly.

C. Conclusions

The doubly labeled water method continues to be a valuable tool for examining energy requirements of soldiers in garrison as well as the various training regiments of military personnel. Energy expenditure during the garrison phase of the 75th Regiment Study, at 4200±800 kcal/d, was considerably higher than expected. Likewise, the 4800±900 kcal/d observed during the FTX was also quite high. As we have observed previously, the energy expenditure during Ranger Training is quite high, with energy expenditures near 5500 kcal/d during 3 of the 4 periods, and just under 5000 kcal/d during the other period. Even higher energy expenditures were observed in the Norwegian Ranger study, with a mean of 5650±800 kcal/d. In addition, there were two women in

this study, with mean energy expenditures of 4750±250 kcal/d, a value comparable to that observed in men during many FTXs. The mean energy expenditure of the men undergoing this same exercise was considerably higher, at 6100±570 kcal/d. However, when adjusting for differences in body size by simply dividing energy expenditure by body weight, the women had a mean value of 81±4 kcal/kg, compared to 78±7 kcal/kg in the men. Therefore, in this small group, it appears that energy expenditure of women and men undergoing the same activities are similar when compared on a body weight basis. However, this would need to be studied in much greater detail before that conclusion can be made.

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III. Stress, Nutrition and Mental Performance

A. Introduction

There have been several changes in personnel on this project during the year. Igor Rybkin MD left the group at the end of June to join the graduate program at the Medical School in San Antonio, TX. His position was filled by Xiaolang Yan, a native of China who has a BS in Medicine from China and a Masters Degree in Nutrition from the University of Colorado. Leigh Anne Howell returned from maternity leave in November and is working with Joe Zhou on the project designed to identify genetic markers for stress responsiveness. Tiffany Mitchell, an AALAS accredited animal technician, joined the laboratory in February to assist Ruth Harris with her experiments. Alan Cheshire returned to Tulane after spending the summer as a student worker assisting several investigators with their projects. Sheila Moore also completed a successful 8 weeks as a NASA SHARP PLUS student in the laboratory, this was her second summer here. Dr. Roy Martin, Professor and Chairman, Department of Foods and Nutrition, University of Georgia, spent a three month sabbatical in this laboratory, working with Gennady Smagin on a study to demonstrate the involvement of central CRF and UCN on restraint induced weight loss in rats. Three members of his laboratory also spent a week here in December and learned procedures for ventricular cannulation and microdialysis from Gennady Smagin.

All of the members of the laboratory attended Neuroscience meetings in New Orleans, presenting 5 posters. Ruth Harris submitted two ROI grant applications, Gennady Smagin submitted an R29 application and You Zhou submitted an RO1 application to NIH. Ruth Harris has been asked to join the editorial board of American Journal of Physiology, Metabolism and Endocrinology.

B. Body

Operant Behavior In Stressed Rats Supplemented With Tyrosine
Bradley Youngblood

In an initial experiment we found that rats exposed to 15 minutes of restraint plus partial water immersion (RWI) stress significantly suppressed response rate and reinforcers earned in rats trained to an FR5 schedule but the stress did not affect free feeding food intake. Injection of either saline or 100 mg/kg L-tyrosine 30 minutes before 15 minutes of RWI stress did not prevent the stress-induced suppression of operant behavior and there was no effect of stress or tyrosine on any monoamine or metabolite measured in the striatum. In a second experiment we investigated whether tyrosine injected immediately after RWI stress would attenuate the stress induced suppression of operant motor performance.

Male Sprague Dawley rats were food restricted to 85% of their normal body weight and trained to a fixed ratio-5 (FR-5) schedule of food reinforcement in a 3 cycle 10-minute time-out, 10-minute time-in procedure. Once the rats met criteria for stable FR-5 performance, they were exposed to 15 minutes of RWI stress then immediately injected i.p. with either saline or 100 mg/kg l-tyrosine prior to placement in the operant chambers. The number of food reinforcers earned was recorded during each 10-minute time-in cycle and response rate was calculated. The number of reinforcers earned during cycle 1 was significantly reduced by tyrosine ($P< 0.02$) and RWI stress reduced the 20 minute cumulative reinforcement by an amount that was close to statistical significance ($P< 0.06$. Response rate during cycle 1 was significantly decreased by tyrosine ($P< 0.02$) and the 20 minute cumulative response rate was decreased significantly by RWI stress ($P< 0.05$). After a week of recovery rats were tested in a 3-cycle free feeding procedure and cumulative 20 and 30 minute food intakes were significantly reduced by RWI stress ($P< 0.04$; $P< 0.01$). Rats were allowed a week of recovery before they were again exposed to 15 minutes of RWI, then injected i.p. with 100 mg/kg tyrosine or saline, placed in the operant chambers for 3-cycles and then killed. Striatal norepinephrine was decreased ($P< 0.058$) by RWI stress. Dopamine, serotonin and their metabolites were not significantly different in RWI stressed rats compared with controls.

Conclusions

Injection of tyrosine immediately after RWI stress did not attenuate the stress-induced suppression of operant response rate but in fact significantly decreased response

rate in non-stressed rats during the first 10 minute cycle and significantly reduced their number of reinforcers earned during cycle 1. Free feeding intake during cycle 2 was significantly reduced by tyrosine but the 20 and 30 minute cumulative intakes were affected by RWI stress but not by tyrosine. It is possible that i.p injection was an additional stressor, producing acute pain and affecting free feeding. The stress-related reduction in striatal norepinephrine concentration may have a role in the depression of operant behavior measured as FR-5 response rate. We are not pursuing this project as we are focusing our efforts on the four major projects, described below.

CRF Receptors, Stress and Behavior
Gennady Smagin

CRF is regarded as the primary mediator of behavioral and physiological responses to stress. Numerous studies suggest that hypersecretion of CRF in response to stress may be a key contributing factor in depression, and that blockade of CRF receptors in the CNS may be useful in treating this disorder (Arato et al., 1986; Nemeroff et al., 1991; Owens and Nemeroff, 1991; Owens et al., 1991). The CRF system is also involved in the regulation of food intake and energy metabolism. CRF treatment induces an increase in the activity of SNS concurrently with a reduction in food intake (Brown and Fisher, 1990; Egawa et al., 1990). The objective of these experiments was to evaluate the involvement of CRF_1 receptors in stress-induced anxiety behaviors and CRF mediated inhibition of food intake.

To investigate the involvement of CRF_1 receptors, a CRF_1 receptor antagonist, NBI 27914, was used. This compound, provided as a gift by Neurocrine, has high affinity to CRF_1 receptors and does not cross react with CRF_2 receptors. Systemically administered, NBI 27914 is able to cross the brain-blood barrier and has been shown to attenuate swim-stress induced elevation of plasma ACTH (Lorang et al., 1997).

Experiment 1:

In this experiment, the effect of NBI 27914 was evaluated in an animal model of anxiety: defensive withdrawal behavior. In the defensive behavior paradigm animals were familiar with the behavioral apparatus. Defensive withdrawal was conducted as described previously (Smagin et al., 1996). The apparatus consists of a 1 meter open field marked with 20 x 20 cm squares. A cylinder (12 cm diameter, 17 cm long), closed at one end is placed in the center of one side of the field. To start the test, the rat was placed in the cylinder. Behaviors scored included: time (latency) to emerge from the cylinder; the times spent inside and outside of the cylinder; locomotor activity (number of lines crossed in the open field). Activity and movement of animals was registered and analyzed automatically (EthoVision, Noldus Information Technology, The Netherlands). Animals were injected with NBI 28914 or vehicle and immobilized in commercial plastic restraining tubes. It has been shown that a short period (20 min) of immobilization induces defensive withdrawal (Smagin et al., 1996; Yang et al., 1990). Immobilization of animals induced defensive withdrawal, indicated by an increase in the time an

animal spent in the enclosed chamber, and decreased exploratory behavior (rearings). Pretreatment of animals with CRF_1 receptor antagonist, NBI 27914 (5 mg/kg) attenuated the effect of immobilization stress (see Appendix, Figure 1). These results suggest that CRF_1 receptors are involved in the stress-induced behavioral changes observed in this animal model of anxiety.

In the second part of the experiment, defensive withdrawal was induced by icv injection of CRF or UCN. It has been shown previously (Yang et al 1990) that CRF and noradrenergic systems are involved in the mediation of defensive withdrawal behavior. Pretreatment of animals with the CRF receptor antagonist, NBI 27914 (5 mg/kg), significantly attenuated defensive withdrawal produced by 0.25 and 0.5 μg of CRF (see Appendix, Figure 2) but was not effective in attenuating UCN-induced defensive withdrawal behavior (see Appendix, Figure 3).

Experiment 2:

To study the involvement of CRF_1 receptors in CRF-induced anorexia, rats were implanted with icv cannulae in the lateral cerebral ventricle. They were fasted overnight and received icv injection of CRF or vehicle (3 μg in 3 μl, or 3 μl, respectively) 1 hour after pretreatment with CRF_1 antagonist or vehicle. Food intake was measured 1,2, 6, 12 and 24 hours after injection of CRF/vehicle. Rats were tail bled before and 1 and 2 hours after CRF/vehicle infusion for determination of serum corticosterone. Pretreatment of animals with CRF1 antagonist (5 mg/kg s.c.) had no effect on CRF-induced anorexia (see Appendix, Figure 4) or CRF-stimulation of serum corticosterone (see Appendix, Figure 5). A limited dose response analysis will be required to demonstrate whether CRF_1 receptors are involved in regulation of food intake and HPA activity in response to exogenous CRF and/or stress.

Conclusions

These results suggest that a specific CRF_1 receptor antagonist produces anxiolytic properties in the models of stress- and CRF- induced defensive withdrawal. Failure to attenuate the UCN-induced defensive withdrawal implies involvement of other types of receptors in UCN-induced behavioral changes.

Measurement of Tissue Urocortin mRNA by Ribonuclease Protection Assay and Characterization of an anti-urocortin (UCN) antibody

Xiaolang Yan, You Zhou and Gennady Smagin

Urocortin (UCN) is a novel neuropeptide related to CRF in mammals. Rat urocortin was originally cloned from a midbrain cDNA library (Vaughan et al., 1995). *In situ* hybridization studies demonstrated that urocortin mRNA was present in various brain areas including Edinger-Westphal nucleus, supraoptic nucleus, hypothalamus, cerebellum, hippocampus and pituitary (Wong et al., 1996). The physiological role of urocortin is not yet well understood but available information suggests that it may be involved in appetite control and possibly in stress-mediated

behavioral responses (Spina et al., 1996). The localization of urocortin mRNA in peripheral tissues has not been studied, however, urocortin-like immunoreactivity was found in duodenal extracts, indicating possible urocortin expression in the periphery (Vaughan et al., 1995). In order to determine the role played by UCN in stress-induced responses it is necessary to develop appropriate assays for UCN mRNA and protein and to understand the dynamic relationship between stress and UCN

UCN Antibody

We have tested two commercially available antibodies and have determined that the anti-UCN antibody sold by Phoenix Pharm. Inc. (Mountain View, CA) detects UCN (as low as 10 ng when exposing the X-ray film for 20 seconds) but not CRF in a Western blot (data not shown). Preliminary studies also suggest that this antibody can be used for immuno-histochemistry but the assay requires further development. A second antibody, provided by Affinity Bioreagents, did not detect UCN and cross-reacted with CRF in Western blots.

Detection of Urocortin mRNA in Rat Tissues by RT-PCR

Tissues were collected from Sprague-Dawley rats and snap frozen in liquid nitrogen. Total RNA was isolated using Trizol Reagent (GIBCO BRL) following standard procedures. Genomic DNA in the RNA preparation was digested with DNase I (1μg of total RNA was digested by 1 u DNase I in 1x DNase I digestion buffer with 40 u of RNase inhibitor RNasin) for 15 minutes at room temperature. RNA was phenol-chloroform extracted and precipitated with ethanol. Reverse transcription (first strand reaction) was achieved using a reverse transcription system (Promega). Reactions with no reverse transcriptase were used as controls for possible genomic DNA contamination in each RNA sample. Following reverse transcription, PCR was conducted according to the company's protocol. PCR conditions were as follows: 94°C for 5min; 94°C, 60°C, 72°C each for 1 min for 40 cycles.

As shown in Figure 6 (see Appendix), UCN mRNA was expressed in both the brain and the periphery including adrenal, fat, heart, hippocampus, hypothalamus, midbrain and pituitary. No UCN mRNA expression was detected in liver, spleen or kidney. These results demonstrate that UCN mRNA is expressed in various brain regions as reported by others using in situ hybridization techniques. We have shown for the first time that UCN mRNA is also expressed in the peripheral tissues heart, fat, adrenal and muscle.

Southern Blotting of UCN PCR Products

To verify that the PCR products were in fact UCN, a Southern blotting was conducted. PCR products were transferred from agarose gel to a nylon membrane by capillary transfer and the DNA was UV crosslinked. The pre-dried membrane was prehybridized in RapidHyb hybridization buffer for 2h and then hybridized with ^{32}P labeled rat UCN probe at 45°C for 2h. The probe was generated by random priming from a UCN insert. To minimize the nonspecific

signals, the blot was subsequently washed at high stringency (0.1% SSC buffer at 65°C for 10 minutes) and finally exposed to a phosphorimaging screen for 3h.

Figure 7 (see Appendix) shows that all PCR products of the expected size, and corresponding to the rat UCN insert control in lane 2, strongly hybridized to the UCN probe whereas there was no hybridization for PCR products of different sizes. These results demonstrate that the PCR had indeed amplified UCN DNA and hence confirmed the results from the RT-PCR.

Development of RNase Protection Assay for Measuring Tissue UCN mRNA

Sprague-Dawley rats were restrained for 1h and decapitated immediately afterwards. Tissues were collected and total RNA was isolated, as described above. Control RNA was obtained from non-restrained Sprague-Dawley rats.

^{32}P labeled antisense rat UCN RNA was synthesized by in vitro transcription, using T7 RNA polymerase from a linearized cloned UCN template. Unlabeled sense strand RNA was also transcribed by T7 RNA from a clone that carried rat UCN cDNA in the opposite orientation. The sense UCN template had been cloned from rat UCN PCR products using the TA cloning method and the PCR2.1 plasmid (Invitrogen). Sense RNA was used as a negative control in the RNase protection assay and for further quantification of tissue mRNA levels. Radiolabled full-length antisense RNA was isolated by electrophoresis on 5% polyacrylamide 8 M urea gel and the probe was eluted into elution buffer overnight. The gel-purified probe (8×10^4 cpm) and RNA samples (20 ug) were hybridized overnight at 45°C. The RNA-RNA hybrids were isolated by digesting non-hybridized RNA using RNase A/T1 in digestion buffer. The digestion was carried out for 30 minutes and the reaction was stopped by inactivation buffer. Protected fragments were subsequently precipitated. The protected fragments were denatured and separated on a denaturing 5% polyacrylamide 8 M urea gel run for approximately 3h. The gel was then directly exposed to a Phosphorimaging screen overnight.

Figure 8 (see Appendix) showed protected fragments from heart, midbrain and hypothalamic tissue. The location of the protected fragments was determined by the sense RNA control. UCN mRNA appeared to be highly responsive to restraint stress in all three tissues. UCN mRNA was almost undetectable in midbrain from control rats but increased dramatically after 1h restraint. We are currently analyzing more tissue samples from rats that were restrained for different durations to determine the effect of stress duration on UCN mRNA expression.

Apolipoprotein E: A Potential Candidate As Genetic Marker For Stress Susceptibility
You Zhou, David Elkins and Leigh Anne Howell

We have been investigating Apolipoprotein E (ApoE) as a potential genetic marker for stress susceptibility. ApoE protein is a ligand for low-density lipoprotein, very low-density lipoprotein and lipoprotein-related protein receptors and plays an important role in lipid

metabolism by mediating lipoprotein removal from the circulation. Human ApoE is a polymorphic protein encoded by a single gene with three major alleles, ε2, ε3, and ε4, among which ε4 has been linked to a higher risk of late-onset Alzheimer's disease (AD). It has been suggested that the ApoE4 phenotype may be more susceptible to abnormal aggregation of cytoskeletal proteins, leading to neurodegeneration, one of the major neuropathological features that may contribute to memory loss in AD patients. Neurodegeneration has been observed by others in apolipoprotein E (ApoE)-deficient mice at as early as 4 months of age. Mechanisms of responsible for CNS neurodegeneration associated with ApoE-deficiency are still unclear. Accumulation of a β-amyloid like protein found in ApoE-deficient mice at ~4 weeks of age, may induce neurotoxicity as this protein has been shown, in vitro, to cause neuronal cell damage and degeneration. This cell degeneration would disrupt neuronal functional integrity and up-regulate autoimmune activities.

In previous studies, we found no difference between wild type and ApoE knockout mice in open field activities (including novelty response, locomotor activity, and exploratory behavior) following a 20-min acute restraint stress. However, ApoE-deficiency caused a severe impairment of spatial learning in non-stressed animals which prevented any further effects of stress on spatial memory measured in a Morris Water Maze test. Studies described here have further analyzed changes in serum corticosterone levels and in ApoE mRNA expression in the hippocampus and hypothalamus in ApoE deficient and wild type mice following exposure to acute or chronic stress. Additional studies examined the effect of stress on autoantibody titres in ApoE deficient mice and their wild type controls.

Corticosterone Changes in Response to Restraint Stress

ApoE-deficient mice and their wild type controls, aged 4-6 weeks old and weighing 18-20 g , were exposed to two sessions of restraint stress followed by different behavioral tests, as described in last quarterly report. At the end of this experiment, 9 days after the last restraint, the mice that had previously been exposed to chronic stress were restrained for 20 min immediately before decapitation. An additional 24 wild-type mice were divided into 3 groups. In a chronic stress group mice were exposed to a 20-min restraint daily for 3 consecutive days. They were killed a day after the 3rd restraint, along with controls, that had not been handled, and an acute stress group of mice that were restrained for 20 min immediately before decapitation. Trunk blood was collected from all mice and serum samples were stored at -80 ^{0}C until assayed. Corticosterone was analyzed in duplicate with samples from all of the animals run in the same assay, using an RIA kit (ICN Pharmaceuticals Inc., Costa Mesa, CA).

Restraint stress caused a significant elevation of serum corticosterone in mice of both genotypes in Experiment 1 (see Appendix, Figure 9A). The non-restrained mice of both genotypes showed similar levels of corticosterone when exposed previously to open field and to water maze tests. However, when the mice were acutely stressed, with the same stressor used 5 times previously, the ApoE mice showed a more pronounced response than the wild-types (see Appendix, Figure 9B). This indicates that ApoE knockout mice do not adapt to stress, even when they have been exposed repeatedly to a same stressor.

ApoE mRNA Expression in Response to Stress

Total RNAs from mouse hypothalamus and hippocampus were obtained using TriZol reagent (Gibco-BRL) according to the company's protocol. Tissue RNAs (~5 μg per lane) were electrophoresed in 1.2% agarose-formaldehyde gel, transferred onto Hybond-N nylon membranes (Amersham Corp, Arlington Heights, IL) and cross-linked to the membrane by UV radiation. The 358-bp ApoE cDNA fragment described previously was labeled with [α-^{32}P]CTP by random primed labeling. Blots were pre-hybridized for 3 hr and hybridized overnight in presence of 50% formamide at 42 ^{0}C, followed by washes to a final stringency of 0.2 x SSC and 0.2% SDS at 65 ^{0}C. The labeled blots were exposed overnight to a PhosphorImaging Screen and signals were detected by using a PhosphorImaging System (Molecular Dynamics, Inc., Sunnyvale, CA). The blots were re-probed with a 29-base human 28S oligonucleotide probe (Clontec, Palo Alto, CA) which was 5'-end labeled with [γ-^{32}P]ATP using a 5'-DNA terminus labeling system (Gibco-BRL). After a 30-min pre-hybridization in RapidHyb buffer (Amersham Corp) at 42 ^{0}C, the membranes were hybridized in fresh RapidHyb buffer containing the labeled oligonucleotide probe for 2 hr at 42 ^{0}C. The membranes were washed twice at room temperature for 20 min in 2x SSC, 0.2% SDS, followed by a 5 min wash at 42 ^{0}C in 0.2x SSC, 0.2% SDS, and re-exposed to the PhosphorImaging screen overnight. The relative level of ApoE mRNA expression was determined as a density ratio of ApoE mRNA to 28S rRNA within the same lanes. The normalized data were analyzed by two-way ANOVA.

ApoE mRNA expression in hypothalamus and hippocampus from mice described above was measured to determine whether ApoE was associated with stress-induced changes of HPA and hippocampal activities. ApoE mRNA expression increased in the hypothalamus of mice killed 24 hours after 3 consecutive days of 20 min-restraint (see Appendix, Figure 10). ApoE mRNA expression in mice exposed to acute stress immediately before decapitation was elevated compared with controls, but the difference did not reach statistical significance. A limited ($p=0.057$) increase in ApoE mRNA expression was also observed in the hippocampus of mice exposed to chronic stress (see Appendix, Figure 10).

Autoantibodies in ApoE Deficient Mice

We have previously reported an altered corticosterone response and increased apoptotic neuronal cells in the cortex of the brain in chronically stressed young ApoE-deficient mice (~12 weeks old when the serum and tissues were collected). In order to determine what mechanisms are involved in this apoptotic process and in the abnormal neuroendocrine activity in ApoE-deficient mice, we have studied the autoimmune reactivity of the sera from wild type or ApoE deficient mice that had been exposed to repeated bouts of restraint stress, as described above. The results from standard ELISA of these sera, using whole brain homogenates of wild-type mice as antigens, demonstrated a higher titer of anti-brain autoantibodies in the sera from ApoE-deficient mice exposed to chronic stress, than in sera from wild-type mice exposed to a similar stress (see Appendix, Figure 11). All the sera were further tested on whole brain homogenates

by Western blot analysis. SDS-PAGEs were carried out using BioRad Mini-gel/Mini-blot systems and Multiscreen apparatus. Prep-gels with 1-well comb loading of brain protein samples were used and all sera were diluted by a 1:40 ratio. The sera from ApoE-deficient mice detected 2 major bands with the molecular mass of ~72 kDa and ~48 kDa respectively (see Appendix, Figure 12A). The ~48 kDa polypeptide was also recognized by sera from some wild-type mice, although a stronger signal was revealed by sera from mice exposed to chronic stress (only 2 of the 15 sera of wild-type mice were shown). None of the sera from wild-type mice detected the 72-kDa autoantigen that was recognized by the sera from both control and stressed ApoE-deficient mice (see Appendix, Figure 12B). Tissue distribution of the anti-72kDa antibody was carried out by Western blotting analysis using the sera from ApoE-deficient mice (see Appendix, Figure 12B). The ApoE sera appeared to detect a different antigen (~75 kDa) in non-brain tissues.

Localization of Autoantigens Detected by the Sera of ApoE-Deficient Mice

To determine the cellular localization of the major components recognized by the sera from ApoE-deficient mice, immunofluorescence microscopy was performed on frozen sections of brain from a wild-type mouse, using the serum from a stressed ApoE-deficient mouse. Briefly, frozen brain sections (8-10 μm thick) were fixed in 4 % paraformaldehyde in PBS for 10 min at room temperature and extracted for 10 min in PBS containing 0.1% Triton X-100, followed by a 5-min incubation in cold methanol (-20 ^{0}C). After 30-min "blocking" with PBS containing 0.05% Twen-20 (PBST) and 3%-BSA, sections were incubated with mouse serum (1:20 dilution in PBST-BSA) and then with FITC-conjugated F(ab')$_2$ fragments of donkey anti-mouse IgG (Jackson ImmunoResearch Inc., 1:100 dilution), for 1 hr each. The sections were washed with PBS, mounted, and examined with a Leica confocal laser scanning microscope. As shown in Figure 13 (see Appendix), strong immuno-reactivity of the serum with neuronal cells was observed in different brain areas, including frontal cortex (A), hippocampus (B) and hypothalamus-cortex regions (C). The major components detected by the sera from ApoE-deficient mice were localized to the nuclei and the neuronal fiber-like structures which were shown at higher magnification in the inserts in (A) and (B), respectively. Such reactivity was not detected by the serum from a stressed wild type mouse (D).

Conclusions

The results from these experiments suggest that ApoE phenotype may be a marker for stress responsiveness. ApoE knockout mice do not adapt to repeated exposure to stress, experience a significant amount of apoptosis in brain tissue and produce an increased titre of autoantibodies. Some of these antibodies are specific for brain antigens and may be responsible for the neurodegeneration observed in these animals. Characterization of the antibodies and their antigens will elucidate the mechanisms responsible for early neurodegeneration in these mice and may provide valuable insight into the mechanisms responsible for Alzheimers disease.

Glucose Utilization in Rats Exposed to Restraint Stress

Jun Zhou, Xiaolang Yan and Ruth Harris

In previous studies, we found that both a single 3 hour restraint and repeated restraint (3 hours/day on 3 consecutive days) caused rats to reduce food intake and body weight. The reduced intake was not compensated for by overeating after the stress, which is the response that is usually observed in food restricted and starved rats once the food restriction is ended Harris et al, 1986). In one study we found that monoamines in various brain regions and of Neuropeptide Y (NPY) protein concentrations in the PVN and NPY mRNA in hypothalamus did not demonstrate any change that could account for the reduction of food intake and body weight in restrained rats (Rybkin et al., 1997). The objective of this project is to determine whether changes in peripheral energy utilization cause erroneous signals to be sent to the brain and result in the stress-induced changes in food intake and body weight. Our first experiment measured glucose utilization of control rats, restrained rats and rats pair-fed to the restrained rats. We also measured insulin-sensitive glucose transport in adipose cells and soleus muscle and fatty acid utilization in adipocytes from rats exposed to repeated restraint.

Oral Glucose Tolerance Test

Thirty six adult, male Sprague-Dawley rats, weighing 325-350g, were obtained from Harlan Sprague Dawley (Houston, TX). They were individually housed in stainless steel cages in a humidity and temperature controlled room (22+2°C 65-75%) on a 12h:12h light dark cycle. The rats were fed low fat diet for 6 days and then divided into two groups: one group remained on low fat diet for another 7 days and the other group was fed high fat diet for the same period (see Appendix, Table 1 for diet composition). Both dietary treatments were further divided into two weight matched groups, one was control and one was exposed to repeated restraint. The high fat restraint group (HFRS) and low fat restraint group (LFRS) were restrained in perspex restraining tubes (Plas Labs, Lansing, MI) for 3 hours in the morning for consecutive 3 days. The high fat control (HFC) and low fat control (LFC) were moved to the same room without access to food or water for the period of restraint.

One day after the end of restraint stress, all the rats were food deprived for about 5 hours and a small amount of blood (300~400ul) was taken by tail bleeding. Immediately after the first tail bleeding, each rat was gavaged with glucose solution (2.5 g/kg). Then additional blood samples were collected 15, 30, 45, and 60 minutes after glucose administration. The blood samples were centrifuged and serum insulin (Rat Insulin RIA kit, Linco Inc, St Louis MO) and glucose (Sigma Diagnostic kit 510. Sigma Chemical Co., St. Louis, MO) were measured. Five days after the end of restraint the rats were killed for determination of body composition, as described previously (Harris and Martin, 1989).

The serum insulin and glucose levels measured during the glucose tolerance test performed one day after the end of stress are showed in Figure 14 (see Appendix). The serum

glucose levels were the same in all four groups, however, serum insulin levels in restrained rat were significantly lower than those of control rats in both high and low fat diet groups. The stress-associated reduction in insulin release was exaggerated in rats fed high fat diet ($P<0.01$) due to the control animals being insulin resistant. The stress-induced change in insulin release was also observed in rats fed low fat diet, but it barely reached statistical significance($P=0.057$). The decrease in glucose-stimulated insulin release of stressed rats, despite similar rates of glucose clearance, indicate that repeated restraint substantially enhances whole-body insulin sensitivity of the rats.

Glucose Transport in Muscle Tissue and Adipocytes

Thirty adult, male Sprague-Dawley rats, weighing 325-350g, were obtained from Harlan Sprague Dawley (Houston, TX). They were individually housed in stainless steel cages in a humidity and temperature controlled room (22+2°C, 65-75%) on a 12h:12h light dark cycle. Daily body weights and food intakes were recorded throughout the experiment. The rats were fed high fat diet for 11 days and then divided into three groups. One group was exposed to repeated restraint, as described above. The second group of rats were fed the amount of food that was eaten voluntarily by the restrained rats. The third group was a control group.

On the first day after the end of restraint stress, all the rats were food deprived for 4~6 hours and then anaesthetized. The soleus muscle from each hind leg was taken immediately for muscle glucose uptake measurement. The epidymal fat was dissected, weighed and digested for isolation of adipocytes which were used to measure glucose transport in the presence of increasing concentrations of insulin. The blood was collected for measurement of serum insulin (Rat RIA kit; Linco Inc.) , glucose (Sigma Diagnostic kit 510), corticosterone (Corticosterone RIA: ICN Biochemicals) and leptin (Rat Leptin RIA kit: Linco Inc.). The carcass was analyzed for body composition.

Glucose Transport in Soleus Muscle

Two pieces(20 - 30 mg) of each soleus muscle from each rat were cut from the outer edges of the muscle to provide four muscle samples per animal. All incubations were performed at 30°C with shaking and a continuous supply of gas (95% O_2,/ 5% CO_2) The four samples from each rat were used for measurement of basal and insulin stimulated 2-deoxyglucose (2DG) uptake. The insulin (Humulin R: Eli Lilly Corp.) was added to preincubation (1X Krebs buffer, 10 mM HEPES, 2 mM sodium pyruvate, 5 mM glucose, 23 mM mannitol, pH 7.5), wash (1X Krebs buffer, 10 mM HEPES, 2 mM sodium pyruvate, 28 mM mannitol, pH 7.5), and transport (1X Krebs buffer, 10 mM HEPES, 2 mM sodium pyruvate, 26 mM mannitol, 0.5 uCi/ml ^{3}H-2-DG, 0.01uCi/ml ^{14}C-mannitol) media at concentrations of 0.25, 0.5, or 2 mU/ml media. The samples were preincubated for 10 minutes and washed for 10 minutes. The sample then incubated in transport media for another 10 minutes and the 2-DG transport was stopped by transferring the tissue to ice-cold saline. Samples were dissolved in 1N NaOH at 90°C, transferred to scintillation vial and ^{3}H-2DG and ^{14}C-mannitol were counted. 2-DG incorporation,

corrected for extracellular fluid volume, was expressed as pmol glucose incorporated per mg muscle.

Glucose Transport in Adipocytes

Adipose cells were isolated from epididymal fat by the method of Rodbell (1964) and suspended in wash buffer (1X Krebs, 0.1mM glucose, 2% BSA). Glucose uptake was measured in basal and insulin (0.1 mU or 0.8 mU insulin /ml) stimulated conditions. 1ml of each cell suspension was added to 2 ml media (1.5 X wash buffer, 0.1 uCi/ml ^{1}4C-mannitol) and incubated for about 30 minutes at 37°C with shaking. Cell number was determined by fixing an equivalent aliquot in osmium tetroxide and counting by Coulter Counter, as described previously (Harris and Martin, 1986a). Then 0.2 mM 2-DG, 1.0 mCi/mM ^{3}H-2DG was added and the sample was incubated for exactly 2 minutes. 200 ul aliquots of the sample were transferred to vials containing 100 ul phthalic acid dinonyl ester and immediately centrifuged to separate cells from media. All incubation conditions were carried out in duplicate and triplicate estimations of transport were made for each incubation. The cell fraction was counted for 2- DG incorporation, and corrected for extracellular fluid volume. Results are expressed as pmol glucose incorporated per cell.

Data Analysis

Body weights, food intakes, glucose tolerance, muscle and adipocyte glucose uptake data were analyzed by repeat measurement. (SAS 6.12 mixed model) The other data were analyzed by two-way ANOVA.(SAS 6.12)

Daily body weights and food intakes of the rats are shown in Figure 15 (see Appendix). As expected, restrained rats reduced food intake and lost body weight during the days of repeated restraint. Pair fed rats lost the same amount of body weight even though they ate less than the RS group, due to spillage, indicating an increased efficiency of energy utilization in these animals.

Figure 16 (see Appendix) shows the results of muscle glucose transport measurements. Basal glucose uptake was the same in all three groups and insulin stimulated glucose uptake in all three groups. However, the degree of insulin stimulation was not the same, indicated by a significant interaction between treatment and insulin (P=0.03) More specifically, glucose uptake was significantly lower in the RS group than control or pair fed groups in the presence of 0.5mU/ml insulin.

Adipocyte glucose uptake is shown in Figure 17 (see Appendix). Basal and insulin stimulated glucose uptake were significantly lower in RS rats than in control or pair fed animals (P=0.03), and there was no difference between control and pair fed groups. The ability of insulin to stimulate glucose uptake was also calculated as percentage of basal levels and the results are showed in Figure 17 (see Appendix). Regardless of the absolute rate of uptake, the

percentage change in glucose transport in cells exposed to insulin was the same in RS and control groups, but was significantly greater in pair fed animals (P=0.03).

Control and RS rats had the same levels of serum insulin, glucose, corticosterone, and leptin. Pair fed rats had lower levels of all these hormones compared with control or RS rats. Some of these measurements reached, or almost reached, statistical significance (Insulin: P=0.07 Glucose: P=0.09 Corticosterone: P=0.04, data not shown). Body composition of the rats is summarized in Table 2 (see Appendix). The results indicated that even though pair- fed and RS rats lost body weight, their body fat content was the same as that of control rats and all of the weight loss was associated with water and protein.

Adipocyte Fatty Acid Utilization

Thirty male Sprague Dawley rats were housed as described above and fed the high-fat diet described in Table 1 (see Appendix) for 11 days. At the start of restraint pair fed rats were fed the amount of food that was eaten voluntarily by the restraint rats each day from the first day of the restraint until the end of experiment. For restraint stress, rats were placed in perspex restraining tubes (Plas Labs, Lansing, MI) for 3 hours in the morning for 3 consecutive days. The control rats and pair fed rats were moved to the same room without access to food or water for the period of restraint. The body weights and food intakes of the rats were recorded daily. One day after the last restraint stress, the rats were food deprived for 4-6 hours and then sacrificed. The blood samples were centrifuged and serum insulin, glucose, corticosterone , leptin and non-esterified fatty acid were measured. The epididymal fat was dissected, weighed and digested for isolation of adipocytes, which were used to measure fatty acid oxidation and esterification in the presence of increasing concentrations of insulin. The whole liver was dissected, weighed and analyzed for lipid and glycogen measurement. Body composition was determined.

Adipose cells were isolated from epididymal fat by the method of Rodbell and suspended in wash buffer (1X Krebs, 5mM glucose, 2%BSA). Fatty acid oxidation and esterification were measured in basal and insulin (0.3mU or 1.5mU insulin/ml) stimulated conditions with triplicate determinations. 0.5ml of each cell suspension was added to 1.5 ml media (1.33X Krebs bicarbonate buffer, pH 7.5, 5.0mM glucose, 2.0% BSA, 0.3uCi/ml ^{14}C palmitate). Cell number was determined by fixing an equivalent aliquot in osmium tetroxide and counting by Coulter Counter, as described previously (Harris and Martin, 1986). The flasks then were gassed with 97%O_2/5 % CO_2, sealed with rubber stoppers carrying center wells, and incubated for exactly 2 hours at 37°C with shaking. The reaction was stopped by adding of 0.5ml 0.5M H_2SO_4 to media and CO_2 was collected by addition of 0.2ml 1.0M benzethonium hydroxide to the center well. The media and cells then transferred to 5 ml Dole's solution (5 parts isopropanol, 1 part Heptane, 0.1 part 0.5M H_2SO_4) and extracted for esterified fatty acid, as described previously (Harris and Martin, 1986).

The body weight and food intake changes were similar to those seen in previous experiments (data not shown). Repeated measurement analysis indicate that body weight was significantly reduced, compared with controls, in both restrained and pair-fed rats. Food intake of restrained rats was significantly lower than controls from the second day of stress and did not return to control levels by the end of experiment.

The fatty acid oxidation results are shown in Figure 18 (see Appendix). The interaction of insulin and treatment was significant ($P<0.05$). Fatty acid oxidation was higher in restrained and pair fed groups in basal conditions ($P<0.02$) and in the presence of 0.3mU insulin ($P=0.02$). But at the very high insulin concentration, the fatty acid oxidation was the same for all three groups. The ability of insulin to stimulate fatty acid oxidation, expressed as % change from basal level, also showed a significant interaction between the treatment and insulin dosage. More specifically, fatty acid oxidation in adipocytes from control rats was stimulated by insulin while cells from restraint and pair fed groups failed to respond, due to relatively high rates of fatty acid oxidation in basal conditions. There were no differences in esterification of fatty acids, or de novo lipogenesis, among the three groups (data not shown).

The serum assay results are summarized in Table 3 (see Appendix). Briefly, the serum hormone levels were the same in control and restrained rats, but pair fed rats had low insulin and leptin concentrations. Serum glucose, free fatty acids, and liver lipids and glycogen were the same in all three groups. The body composition of rats measured one day after the end of restraint indicated that lean body mass was the only significant loss and there was no significant change in body fat or ash of restrained or pair-fed rats, compared with controls.

Conclusion

Our results indicate that repeated restraint stress has a great effect on peripheral tissue nutrient utilization and these effects can last a long time after the stress has finished. The body weight loss is not simply due to reduced food intake, but also results from metabolic changes caused by the shifts in relative concentrations of anabolic and catabolic hormones. The increased adipocyte fatty acid oxidation may explain body fat content remaining constant despite a dramatic reduction in adipocyte glucose transport in animals exposed to repeated restraint stress. Future studies will determine liver glucose and fatty acid utilization and its role in determining metabolic responses to stress.

Involvement Of Central CRF Receptors In The Regulation Of Food Intake And Body Weight Of Rats Subjected To Repeated Restraint Stress

Gennady Smagin, Ruth Harris, Leigh Anne Howell

We have established that repeated restraint stress inhibits the food intake and produces weight loss in animals (Harris et al, 1998). However, the specific mechanisms responsible for the effects of repeated restraint on food intake and energy metabolism are not clear. Stress can be defined as activation of cerebral corticotropin-releasing factor (CRF) system, which triggers

activation of hypothalamic-pituitary-adrenal axis, central catecholaminergic and other peptidergic systems (NPY, vasopressin and others). The CRF system is very complex and involves interactions between CRF, urocortin, CRF binding protein and CRF receptors. It is known that CRF and urocortin have appetite suppressing properties and may be expressed/released during stress (Spina et al., 1996). It has been shown previously that administration of a CRF antagonist can attenuate the acute stress-induced decrease in food intake (Krahn et al, 1986) . The present studies were designed to determine whether the central CRF system is involved in mediation of repeated restraint stress-induced changes in food intake and energy metabolism.

Lateral Ventricle Infusions of a CRF Receptor Antagonist

Adult male Sprague Dawley rats were implanted with cannulas in the lateral cerebral ventricle and allowed to recover from surgery. After the period of recovery, they were adapted to a liquid diet, and baseline data for daily food intake and body weight were recorded for 7 days. Four experimental groups were formed: saline/quiet, saline/stress, CRF antagonist/quiet, CRF antagonist/stress. A mixed antagonist of CRF receptors, α-helical CRF_{9-41} was injected icv 10 min prior the stress (50 μg in 3 μl), control animals received 3 μl of saline. Animals in the stress groups were stressed in commercial plastic restrainers for 3 hours in the morning (8:00-11:00 am). Blood samples were taken by tail bleeding 1 hour after infusions/beginning of stress. Immediately after restraint, animals were transferred into their home cages and received liquid diet. Food intake was measured at 2, 4 and 7 hours after stress (light phase), 1, 2 hours of dark phase (8 and 9 hours after stress) and 24 hours after stress. The same measurements were made on the 2nd and 3rd days of restraint. Body weights and food intakes were measured for four more days. The animals were killed by decapitation, blood was collected for serum analysis and the carcasses were analyzed for composition.

Prior to restraint, the four groups of animals maintained similar food intakes with a constant rate of growth. Animals in the sal/quiet and CRF antagonist/quiet groups decreased their rate of growth during the experimental period due to the stress caused by injections/excessive handling. Repeated restraint of animals that received saline infusions, caused a significant loss of weight, and body weight remained significantly different from the sal/quiet group throughout the rest of the experiment. Administration of α-helical CRF attenuated the weight loss caused by stress on the first and second day of restraint. There was no significant difference in weight of the two stressed groups by day 3 of restraint (see Appendix, Figure 19).

On day one of stress, administration of α-helical CRF attenuated the acute effect of stress on food intake during the 2 hours immediately after the end of restraint. The effect was not significant at any other period of the light or dark phases. The reduction in 24 hour food intake of restrained rats was significantly attenuated by the CRF receptor antagonist, and there was a significant interaction between treatment and infusion for the 24 hour food intake period (see Appendix, Figure 20A). On the second day of stress, there was a significant effect of stress

on cumulative food intake during dark phase and total 24 hour food intake. However, there were no significant effects of the CRF receptor antagonist or stress on food intake in any of the shorter time-intervals measured (see Appendix, Figure 20B). On the final day of restraint, there were no significant effects of the CRF receptor antagonist on food intake in any periods of observation. However, there was an overall effect of stress on food intake for the dark period and for 24 hours (see Appendix, Figure 20C).

Administration of a-helical CRF did not attenuate the effects of stress on serum corticosterone (see Appendix, Figure 10) which suggests that infusion into the lateral ventricle did not prevent activation of the HPA axis. Analysis of serum (see Appendix, Table 4) collected from the animals 4 days after the end of repeated restraint showed that both groups of rats that had been infused with a-helical CRF had substantially elevated corticosterone concentrations which were higher in the stressed than control animals, suggesting that the high concentration of receptor antagonist that had been used in this study had chronic effects on activation of the HPA axis, possible to compensate for inactivation of the CRF receptors or CRF binding protein. This elevation in corticosterone was associated with a small, but significant, increase in serum insulin. Body composition of the rats, shown in Table 4 (see Appendix), indicated that the receptor antagonist had reduced the amount of weight lost by stressed rats, compared with their controls and that, as in previous studies, the majority of the weight loss was due to a change in body water. There were no differences in carcass fat or protein content of any of the groups of rats.

These results suggest that administration of a CRF receptor antagonist into the lateral ventricle prior to restraint attenuates the effects of stress on body weight and food intake on the first day of repeated restraint but that the effect diminishes with repeated exposures to stress. As the restraint-induced activation of the HPA axis was not prevented in this experiment the next study determined whether infusion of the antagonist into the third ventricle inhibited stress-activation of the HPA axis and the associated reductions in food intake and body weight.

Third Ventricle Infusions of a CRF Receptor Antagonist

Adult male Sprague Dawley rats were implanted with cannulas in the 3rd cerebral ventricle and allowed to recover from surgery. They were adapted to a high fat diet (40% kcal fat, see Appendix, Table 1) and daily body weights and food intakes were recorded throughout the experiment. Four experimental groups were formed: saline/quiet, saline/stress, CRF antagonist/quiet, CRF antagonist/stress. Animals in stress groups were restrained in commercial plastic restrainers for 3 hours in the morning (8:00-11:00 am) on three consecutive days. On each day of restraint a mixed antagonist of CRF receptors, α-helical CRF_{9-41} (ahCRF), was injected icv 10 min prior to stress (10 μg in 1 μl), control animals received 1 μl of saline. Blood samples were taken by tail bleeding 1 hour after infusions/beginning of stress on each of the three days for measurement of corticosterone. Immediately after restraint, animals were transferred into their home cages with free access to food. Body weights and food intake were monitored for four days after the end of stress when the animals were decapitated. Blood was

collected for determination of serum hormone concentration, hypothalamus was dissected and snap frozen for subsequent determination of PVN CRF concentration, fat pads and liver were weighed and the carcass was frozen for determination of body composition.

Prior to stress, animals maintained a stable food intake with a constant rate of growth. Weight gain was inhibited in all experimental groups during the period of restraint due to the stress associated with central injections and tail bleeding. Repeated restraint of animals that received saline infusions, caused a significant decrease in body weight, which remain significantly different from the sal/quiet group to the end of the experiment. Administration of ahCRF attenuated the decrease of body weight caused by stress, and body weights of the αhCRF/quiet group and the ahCRF /restraint group were not different throughout the experiment. (see Appendix, Figure 21A) Administration of αhCRF significantly attenuated the stress-induced hypophagia in animals on all three days of restraint (see Appendix, Figure 21B).

Restraint induced a significant increase in serum corticosterone in comparison with control groups and ahCRF infusion did not influence the response, indicating that activation of the HPA axis was not attenuated by this treatment. (see Appendix, Figure 22). Administration of αhCRF produced a significant effect on the weight of inguinal (ING) and retroperitoneal (RP) fat pads, measured 4 days after the end of stress. There were no differences in other organ weights (see Appendix, Table 5).

Conclusions

The results of this study suggest that antagonism of hypothalamic CRF receptors immediately prior to restraint stress inhibits the effects of stress on food intake and body weight independently from activation of the HPA axis. This implies that acute activation of hypothalamic CRF receptors during stress initiates a cascade of events that lead to sustained reductions in body weight. Future studies will investigate the relationship between CRF receptors and peptide mechanisms that influence energy balance and are normally activated by stress.

The Effect Of Two Bouts Of Repeated Restraint On Body Weight, Body Temperature And Serum Cytokine Concentrations

Ruth Harris

In previous experiments we have shown that rats exposed to repeated restraint experience a temporary reduction in 24 hour food intake but maintain a reduced body weight, compared with controls, for long periods of time. The objective of this experiment was to determine whether exposing rats to more than one bout of repeated restraint had any further effect on body weight and also to determine whether the changes in energy balance were associated with a stress-induced increase in circulating concentrations of inflammatory cytokines and fever, as the weight

loss associated with accidental or surgical trauma is accompanied by elevated rates of cytokine release that induce fever and promote tissue catabolism (Lennie et al., 1996, Chance et al, 1987).

Methods and Results

Fourteen male Wistar rats were housed in individual cages with free access to water and a high fat diet that contained 40% kcal fat and 16 % kcal protein (see Appendix, Table 1). After 10 days adaptation to the diet the rats were divided into two weight-matched groups and one group was exposed to repeated restraint (3 hours/day for 3 days). On the morning prior to the start of stress and for the next 5 days a small tail blood sample was collected at a time that was equivalent to 1 hour after the end of restraint (11.00 a.m.) In addition, rectal temperatures of the rats were measured immediately before and immediately after restraint and at an equivalent time on the four days following stress. Food intakes of the restrained rats had returned to control levels within 2 days after the end of stress. Seven days after the end of the first bout of repeated restraint the rats were exposed to a second bout of repeated restraint. Rectal temperatures were measured and blood samples were collected as before. Thirteen days after the end of the second bout of repeated restraint the rats were killed for determination of body composition.

The daily body weights and food intakes of the rats are shown in Figure 23 (see Appendix). The rats lost weight in response to the first bout of repeated restraint and then lost more weight in response to the second bout of restraint. This indicates that they had not habituated to repeated exposure to the same stress. Food intake was inhibited by both bouts of restraint but quickly returned to control values after the end of restraint. Rectal temperatures of the rats measured before and after restraint are shown in Figure 24 (see Appendix). Restraint caused a significant elevation of body temperature that was reversed within hours of the end of restraint so that there was no maintained elevation of body temperature or fever. Serum IL-6 and serum leptin, measured repeatedly during the experiment, are shown in Figure 25 (see Appendix). Although restraint caused a significant elevation of the inflammatory cytokine, IL-6, which was undetectable prior to stress, there was no difference between the levels induced in non-restrained control rats and the restrained animals. These results indicate that, unlike trauma, the weight loss associated with repeated restraint is not exaggerated by the catabolic effects of cytokines which induce fever and protein breakdown. Of more interest was the inhibition of serum leptin, especially following the second bout of restraint. The drop in leptin was not an immediate response to stress as it did not decrease until the second day of restraint, which suggests that it was a secondary, compensatory change in the restrained rats. Leptin was not different between the two groups by the end of the experiment, 13 days after the end of the second bout of restraint. Although the weights of the stressed rats remained lower than those of controls until the end of the experiment, body composition, shown in Table 6 (see Appendix), shows that there was no difference in body fat content of the two groups of animals.

The results from this study provide several useful pieces of information. The first is that the loss of weight in restrained rats differs from that experienced by animals exposed to a more severe trauma, in that there is no significant elevation of inflammatory cytokines beyond that in

control animals and there is no stress-induced fever. The exaggeration of weight loss following the second bout of repeated restraint indicates that the animals had not habituated to the stressor and that repeated exposures have an additive effect on body weight. Finally, the decline in serum leptin concentrations appears to be a delayed response to the stress but may contribute to the shift in body composition of the animals as leptin inhibits fat accretion but protects lean body mass.

The Effect Of Dietary Amino Acids On Spatial Memory In Rats Sleep Deprived By The "Flower-Pot" Technique

Bradley Youngblood, David Elkins, Gennady Smagin and Ruth Harris

We have previously shown that rats that have been sleep deprived by the "flower-pot" technique for 96 hours have an impaired reference spatial memory, represented by Trial 1 in the Morris Water Maze Place Learning Set Task (Youngblood et al., 1997). The stress of sleep deprivation also results in a significant increase in serotonin (5HT) metabolism in several brain regions, including the hypothalamus, hippocampus and brain stem. Brain dopamine and norepinephrine metabolism are not significantly changed by sleep deprivation. Physiological responses observed in rats sleep deprived by this technique include hyperthermia, increased serum corticosterone concentrations, decreased serum insulin and glucose, decreased thymus gland weights, and weight loss despite maintenance of a normal food intake (Youngblood et al., 1997). The objective of these studies was to determine whether we could modulate spatial memory or the physiological responses to sleep deprivation by supplementing the diet with amino acids that influence central concentrations of neurotransmittors involved in the response to stress.

Dietary Supplementation with Valine

In this study rats were fed either a control diet, which contained 60% of the NRC amino acids for growing rats and supported normal growth in the older animals used in this study, or the same diet supplemented with valine to a final concentration of 2.4% of diet by weight and 25% of total dietary amino acids. The diet contained 0.08% by weight tryptophan which represented 1.08% of total dietary amino acids. Our objective was to try and modulate central concentrations of serotonin by limiting availability of its precursor, tryptophan. Valine competes with tryptophan for transport across the blood brain barrier. By modulating central concentrations of serotonin and serotonin metabolism we hoped to determine whether the stress-induced changes in spatial memory of sleep deprived rats were mediated by hippocampal serotonergic pathways.

A total of 48 male Wistar rats were divided into six groups of eight rats with ad libitum access to water and a control liquid diet supplemented with vanillin (7.0 mg/L) to enhance palatability. After 4 days the rats were divided into two groups and one continued to receive control diet while the other received the same diet supplemented with valine. After 2 days each dietary group was further subdivided into three treatment groups: sleep deprived (sd), tank controls (tc) or cage controls (cc). sd rats were housed in water tank cages on a small platform, tc rats were housed in similar cages with a larger platform that permitted the rats to sleep and cc

rats were housed in shoe box cages. On the second, third and fourth days of sleep deprivation each rat was tested in a Morris Water Maze Place Learning Set task which has been described in detail previously (Youngblood et al., 1997). On the morning of the fourth day rectal temperatures of the rats were recorded. That afternoon the rats were decapitated and trunk blood was collected for measurement of serum insulin and corticosterone. The thymus gland was weighed and the hippocampus, hypothalamus, brain stem and frontal cortex of the brain were dissected, snap frozen and analyzed for monamine concentration, as described previously (Youngblood et al., 1997).

The results from the Place Learning Set task on day 2 of sleep deprivation are shown in Figure 26 (see Appendix). On days 2 and 3 of sleep deprivation there was no effect of either diet or treatment on short-term, or working, spatial memory, as indicated by distance traveled to locate the platform during the second trial from each starting point. On Day 2, sleep deprived rats from both dietary groups and the tc rats on valine diet all performed significantly worse than control rats during the first trail from each starting point, indicating a significant impairment of reference, or long-term, spatial memory. There were no differences between any groups on Day 3 of sleep deprivation but by Day 4 there were significant impairments in both reference and working memory of sleep deprived rats from both dietary groups. Reference memory was also impaired in tc rats fed control diet whereas working memory was impaired in tc rats fed the valine supplemented diet.

Table 7 (see Appendix) shows the physiological parameters and brain neurotransmitter levels that were measured in these rats. There was no significant effect of stress on food intake but food intake was reduced in rats fed the valine diet compared with those fed the control diet. This was true for all 4 days of the sleep deprivation protocol. There were no significant differences in serum insulin of the different groups of rats and corticosterone has yet to be measured. Thymus weight was significantly reduced in both tc and sd rats, compared with control rats, and sleep deprived rats were hyperthermic on the 4th day of sleep deprivation. Values for tryptophan measured in the hypothalamus and hippocampus suggested that valine supplementation of the diet had reduced tryptophan in the brains of control rats but this deficit was reversed, or neutralized, by stress. There were no significant effects of stress or diet on hypothalamic or hippocampal 5-HT concentration but its metabolite, 5-HIAA, was significantly elevated in both tc and sd rats, compared with controls. This resulted in a significant increase in serotonin metabolism (5-HT/5-HIAA) in both the hippocampus and hypothalamus of rats in both dietary groups.

These results demonstrate that supplementation of the diet with valine did result in a small decrease in brain tryptophan concentrations but did not influence serotonin concentrations . The limitation of tryptophan transport did not prevent the stress induced increase of serotonin metabolism, suggesting that the increased presence of 5-HIAA is due to a suppression of 5-HT and 5-HIAA clearance, rather than increased 5-HT synthesis. The valine supplemented diet had no beneficial effects on either spatial memory or the physiological response of the rats exposed to sleep deprivation. Therefore, although we did manage to produce a small modulation of brain

tryptophan it is still not possible to determine, whether, or not, the increased rate of serotonin metabolism is responsible for stress-induced changes in spatial memory.

Supplementation of the Diet with Histidine

In a previous study, with rats exposed to restraint stress, we found that dietary histidine, the amino acid precursor for histamine, improved spatial memory of control, but not stressed rats. In that study the diet contained a relatively high concentration of the high quality protein casein, which may have prevented us from inducing any amino acid specific effects on behavior. We have now developed an amino acid diet that provides the minimal amount of amino acids required for normal growth in the rats used in our studies. This diet improves the likelihood of our being able to identify beneficial effects of amino acid precursors of neurotransmitters that effect behavior. In this study we determined whether supplementing the diet with histidine had any beneficial effects on memory or physiology of rats exposed to SD.

Male Wistar rats were purchased from Harlan Sprague Dawley and housed in individual shoe-box cages. The rats were adapted to the amino acid diet for 5 days and were then tested for spatial memory in a Place Learning set task, described previously. Rats that were unable to locate the platform starting from two different locations were not included in the study. The rats were divided into three weight matched groups and each of these groups was subdivided into two, one half received the non-supplemented diet and the other received the same diet supplemented to 4.5%, by weight, histidine. After a further 4 days, one group of rats remained in the shoe box cages as controls, one group was placed in water tank cages with large platforms, as tank controls (TC) and the final group was subjected to sleep deprivation by housing on small platforms in water tank cages. Food intakes and body weights were recorded daily for the 4 days of sleep deprivation. Reference and working memory were tested on the 2^{nd}, 3^{rd} and 4^{th} day of SD in a Morris Water Maze, Place Learning set task, as described previously. On the morning of the fourth day rectal temperatures of CC and SD rats were measured prior to the Maze test. On that afternoon the rats were killed by decapitation, blood was collected for serum analysis, the thymus was dissected and weighed, and the brain stem, hypothalamus, cortex and hippocampus were rapidly dissected and snap frozen for measurement of histamine, acetylcholine and monoamines.

Due to the large number of animals required for this study it was completed with four sets of 12 animals to give a total of 8 rats per group. There was no difference in performance of the two dietary groups in the Place Learning Set Task, when all six sets were considered. The results of the last three sets on day 2 of sleep deprivation are shown in Figure 27 (see Appendix). Supplementing the diet with histidine totally prevented any SD induced impairment of reference memory. On the two subsequent days of sleep deprivation there were no significant effects of stress or diet on memory of the rats, probably due to the large variability between animals as we have previously found a reliable difference between SD and control rats on day 4. Table 8 (see Appendix) shows some of the physiological measures made on the rats. Supplementing the diet with histidine did not prevent the stress-induced loss of body weight, elevation of body

temperature, elevation of serum corticosterone or decline in serum leptin concentrations. Histidine also had an independent inhibitory effect on food intake, consistent with reports that central histamine suppresses feeding in rats (Mercer et al., 1990; Ookuma et al, 1987). The measurements of central neurotransmitters are shown in Table 9 (see Appendix). The histidine supplemented diet caused four-fold increases in histamine concentrations in the hypothalamus, cortex and hippocampus. The increase was only two-fold in the cortex. The histidine diet did not appear to influence stress-associated changes in brain serotonin (5HT) or serotonin metabolism (5HIAA/5HT) .

The results from this experiment demonstrate that we prevented the early decline in reference spatial memory of sleep deprived rats with high dietary concentrations of histidine. The HPLC analysis of brain histamine indicate 4 fold increases in concentration, which is excessive. Therefore, we are initiating a dose response study in which spatial memory of CC and SD rats fed diets containing 4 different concentrations of histidine will be measured.

Dietary Histidine Dose Response Study

In this study we conducted a dose-response study, feeding rats diets of increasing histidine concentration, to determine whether concentrations lower than 4.5% moderated the effect of sleep deprivation on memory of REMd rats. Due to the large number of animals needed for this study, subgroups of rats were tested on a weekly basis for 8 weeks. Each subgroup consisted of 8 rats representing one rat per treatment group. Male Wistar rats were purchased from Harlan Sprague Dawley and house in individual cages. They were adapted to control liquid diet (7.4% amino acids, 0.24% histidine) for 5 days. All rats were tested in the Morris Water maze, given 2 trials from one starting point, to exclude animals that were unable to locate the hidden platform. Animals were then allocated to one of 8 treatments groups: cage control or REMd fed diets containing 0.24 (control), 1.125, 2.25 or 4.5% histidine by weight. After 4 days adaptation to the diet the REMd rats were placed in sleep deprivation cages consisting of a small platform over water. Cage controls remained in shoe-box cages in the same room. Food intake and body weight was recorded daily and rectal temperature was measured on the second day of REMd. On the morning of the second day (48 hours) of sleep deprivation all rats were tested for reference and working spatial memory in a Place learning Set Task in the Morris Water Maze, as described previously. Following testing the rats were returned to their experimental cages until they were decapitated that same afternoon. The brain was rapidly dissected for collection of brain stem, hippocampus, hypothalamus and cortex. Blood was collected for determination of serum hormones and thymus and epididymal fat pads were weighed.

The results from the Place Learning set task are shown in Figure 28 (see Appendix). Distance traveled to find the platform is used as there was a significant difference in swim speed of the animals with sleep deprived rats swimming faster than controls (stress $P<0.001$, Diet, NS, Interaction $P<0.02$). There was a significant effect of both diet and stress and an interaction between stress and diet on distance traveled in trail 1, an index of reference spatial memory (Diet $P<0.009$, Stress $P<0.001$, Interaction $P<0.03$) As shown in Figure 27 (see Appendix), histidine

had an independent negative effect on memory which was not additive to the stress-induced impairment. The majority of the increased distance traveled by rats on the histidine diets occurred in set 1 of Trial 1 which implied that they were not learning the task and were not adjusting their search strategy successfully, this may represent an impairment of immediate or egocentric memory. In Trial 2 there was a significant effect of stress but no significant effects of diet on working memory. Although the diet effect did not reach statistical significance in trial 2 the distance traveled by control rats on the histidine supplemented diet was not any less than that traveled by control rats on the control diet. As shown in Figure 27 (see Appendix), the difference in memory between cage control rats on control diet and those on the histidine diet was also predominantly accounted for by set 1, which would be a measure of working memory.

Fat pad and thymus weights are shown in Table 10 (see Appendix). There was no effect of diet or stress on the weight of epididymal fat. There was a significant effect of stress and of diet on thymus weight, which was reduced in REMd rats compared with controls, and was decreased by supplementation of the diet with histidine, but there was no interaction between diet and stress. The serum assays, brain histamine concentrations and data analysis of food intakes and body weights are in progress.

Conclusions

The results from this and the previous study indicate that supplementing the diet of rats with histidine, the amino acid precursor of histamine, has a significant impact on spatial memory of rats. Histidine has a detrimental effect on immediate learning, as illustrated by the rats inability to find the platform in the early trials of the Place Learning Set task, this may be interpreted as an inability to recognize a failure of strategy and development of alternate searching behavior. In later trails of the test it appears that a high concentration of histidine has a beneficial effect on learning. Sleep deprivation also impairs spatial memory, however, there is no additive effect of diet and stress on spatial memory, suggesting that similar pathways are involved in the response to both dietary histidine and chronic stress.

C. Conclusions

The results of studies described above illustrate the success of using a multidisciplinary team in this task. Our goal is to identify nutritional interventions that have a positive impact on stress-induced behavior. Sleep deprivation is the only model in which we use published literature to identify nutritional strategies that may influence learning behavior. To date, this approach has provided more negative than positive results. The progress made on other projects indicate that a more useful strategy is to identify the neurological and physiological changes that underlie a behavioral response with the intention of using this information to identify dietary manipulations that will impact a defined metabolic pathway.

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IV. Stress, Nutrition and Work Performance

A. Introduction

Nutritional needs of soldiers have been evaluated on a periodic basis since World War II, with responsibility for prescribing nutritional standards for operational (deployment) rations belonging to the Army Surgeon General. Nutrient content of operational rations, to include protein, has been determined by reviewing published research and incorporating the judgment of experts; emphasis is on optimal requirements rather than minimum daily requirements. Since 1947, specific standards for the military have been established, adapted from the Food and Nutrition Board (FNB) Recommended Dietary Allowances (RDA) to allow for additional physical requirements of military activities. After reviewing research on energy expenditure and nutrient consumption of soldiers during World War II, the Army established requirements of 3600 kcal and 100 g protein per day for physically active personnel deployed in temperate climates (AR 40-250, 1947). Additional requirements for environmental extremes were not addressed, and no changes have been made in those requirements since they were initially established.

Soldiers, when deployed or on field training exercises, are expected to perform physical activity at a level that may be well above their normal activity level while in garrison. Although

energy expenditure may be increased, energy intake from foods consumed in the field is less than energy intake in garrison, ranging from 2009-3050 kcal in the field versus 2773-3173 kcal in garrison for men (Baker-Fulco, 1995; Thomas et al., 1995; Cline et al., 1997; Tharion et al., in press); and ranging from 1658-2343 kcal in the field versus 1832-2592 kcal in garrison for women (Edwards et al., 1991; Rose et al., 1989; Hirsch et al., in press; King et al., 1994; Cline et al., 1996).

Energy expenditure has been measured in male soldiers during strenuous field training exercises, ranging from approximately 3400 kcal to over 5000 kcal in various settings, and energy deficits of 520 kcal to almost 2000 kcal per day have been measured when comparing energy expenditure with energy intake during field operations (Tharion et al., 1997; Baker-Fulco, 1995, Shippee et al., 1994).

Protein requirements were established in 1947 for a reference male soldier of 68 kg, with 100 g providing 1.47 g/kg. The current reference weight of soldiers is 78 kg, which would translate to 1.28g/kg of protein. Recent studies on consumption patterns of military personnel have reported an intake of 1.34-1.64 g/kg of protein in garrison, and an intake of 1.14-1.65 g/kg in field operations (Cline et al., 1997; Thomas et al., 1995; Rose and Carlson, 1986; Askew et al., 1986; Morgan et al., 1988;Edwards et al., 1991). Male military personnel maintain high protein intakes from food consumption in garrison as well as during field operations; however, energy intake is reduced in the field. Females generally consume less energy and protein than guidelines during field exercises where access to foods is limited to operational rations.

It is now known that regular exercise increases protein needs, with intakes of 12-15% of energy from protein recommended, unless energy intake is insufficient. Insufficient energy intake can lead to negative nitrogen balance, even at protein intakes that have been promoted positive nitrogen balance at adequate energy intakes (Munro, 1951; Butterfield and Calloway, 1984; Calloway and Spector, 1954; Goranzon and Forsum, 1985; Walberg et al., 1988) Current evidence suggests that strength or speed athletes should consume about 1.2-1.7 g/kg of protein and endurance athletes about 1.2-1.4 g/kg (Lemon, 1991). Inadequate carbohydrate (CHO) intake will cause more rapid depletion of muscle and liver glycogen during exercise and may contribute to greater protein utilization (Anderson and Sharp, 1990; Lemon and Mullin, 1980; MacLean et al., 1989). Because of the established link between CHO intake and optimal performance in high-demand exercise (Costill, 1988), military requirements have been set at a minimum value for CHO (440 g or 48.9 percent of energy) that must be exceeded in field operations. Current studies on military field operations have reported inadequate mean CHO intake, ranging from 244-369 g; energy deficits were also reported for these populations studied (Askew et al., 1986; Morgan et al., 1988; Edwards et al., 1991) with a majority coming from a deficient carbohydrate intake.

Because soldiers are self selecting a diet deficient in carbohydrate (and energy) but maintain protein intake when deployed or on field training exercises, it is of interest to know whether 1) an increased protein content in the rations provided stimulates an increase in total

energy intake, and 2) high levels of protein or amino acid intake help to maintain or improve physical performance under conditions of energy deficiency. We have been conducting a pilot study which, if successful, will help us in the design of future experiments that can address issues of dietary protein requirements/needs under conditions of energy deficiency. The specific objectives of this project are: A) To determine the effects of moderate (750 kcal/day) caloric restriction in combination with daily exercise (500 kcal energy expenditure) on body composition, muscular strength, aerobic (endurance) performance, and anaerobic capacity, and B) to determine the effects of moderate (750 kcal/day) caloric restriction in combination with daily exercise (500 kcal energy expenditure) on protein balance as determined from urinary nitrogen excretion.

B. Body

Experimental Design

This study was designed to address whether moderate caloric restriction (750 kcal/day) in combination with daily exercise (500 kcal energy expenditure) would have detrimental effects on muscular strength, endurance, and anaerobic performance in exercise-trained men and women. Prior to experimental testing, subjects went through a general clinic screening, which included a physical examination with routine blood and urine chemistries. Basal metabolic rate was determined by indirect calorimetry. Body mass index (BMI) and waist-to-hip ratios (WHR) were also be calculated. To determine fitness level, peak oxygen consumption (VO_2) was measured in all subjects during an incremental test to exhaustion while running on a treadmill. Before the start of the study, subjects also completed questionnaires inquiring about physical activity and food preferences. They completed a three day food record which will be analyzed and used as a baseline measure of habitual energy and protein intake. In order to (1) decrease any potential experimental artifact related to individual differences in energy balance and diet composition during the baseline period (week one), and (2) to adjust intake during the period of caloric restriction (weeks two and three), subjects consumed meals designed and prepared by the Pennington Center Metabolic Kitchen and consisted of a protein content fixed at 1.3 grams/ kg body weight. The subjects were instructed to eat no other food and to eat all of the food provided.

The protocol consisted of three weeks of physical training with pre and post caloric restriction measurements of body composition, muscular strength and endurance, endurance performance, anaerobic capacity, and urinary nitrogen balance:

(1) Baseline Period (days 1-7): Subjects reported to the Metabolic Kitchen (MK) for breakfast and dinner meals. Snacks and lunch were provided for off-campus consumption. Daily energy intake followed in accordance with each subject's individual energy needs to prevent weight loss or gain (i.e. eucaloric). In addition, subjects reported daily to the Exercise Testing Facility (ETF) to perform an acute bout of aerobic exercise predetermined to elicit a 500 kcal energy expenditure.

(2) Pre caloric restriction measurements: On day five of the baseline period, subjects completed a muscular endurance test following that morning's running session. On day six of the baseline period, subjects reported to the ETF in the afternoon for muscle strength and anaerobic performance tests. These tests were performed at least four hours after that day's treadmill exercise. On day seven of the baseline period, subjects reported in the morning (prior to breakfast consumption) to the clinic for body composition analysis. Lean body mass and percent body fat were determined by dual energy x-ray absorptiometry (DEXA). In place of that morning's treadmill exercise bout, subjects participated in an endurance performance test. On days 6 and 7 all urine excreted was collected and divided into two 24-hour periods for an assessment of daily urinary nitrogen balance.

(3) Caloric Restriction Period (days 8-21): Subjects continued to report daily to the ETF for their exercise sessions. Each bout was of sufficient intensity and duration to elicit a 500 kcal energy expenditure. Subjects continued to report to the MK for their daily meals, however their total daily energy intake was reduced by 750 kcal/day. A majority of the energy deficiency came from withholding carbohydrate, while the protein intake was held constant at 1.3 g/kg body weight. However, the percent of calories which come from protein increase above the baseline period as total energy intake was reduced. On day 19 of the energy restrictive phase, subjects repeated the muscular endurance test following that morning's running session. On day 20 of the energy restrictive phase, the muscle strength and anaerobic performance tests were repeated and conducted as described above. On day 21, body composition by DEXA was re-assessed, and in place of the final treadmill exercise bout, subjects repeated the endurance performance test. On days 20 and 21 all urine excreted was collected and divided into two 24-hour periods for a re-assessment of daily urinary nitrogen balance.

A separate group of individuals matched for gender, body composition, fitness level, and age will serve as a control group. These subjects will follow the protocol described above, with one exception: days 8-21 will continue <u>without caloric restriction</u>, i.e., control subjects will continue to consume the eucaloric diet established during the baseline period. During recruitment, subjects were randomly assigned to either the control or the experimental (calorie restricted) group.

(4) Subjects: Exercise trained, healthy male and female volunteers (18-35 yrs) were studied, and an attempt was made to recruit an equal number of men and women into both the experimental and control groups. Exercise trained was defined as (1) participation in regular aerobic activity for the last six months with the average weekly amount of work equaling at least 3 but no more than 5 hours per week, and (2) in men, a peak VO_2 of between 45 to 60 ml/kg/min, and in women, a peak VO_2 of between 40 to 55 ml/kg/min. Healthy was defined as (1) the absence of cardiovascular, metabolic, endocrine, neuromuscular disease, and (2) in men, a body fat percentage between 8 and 20%, and in women, a body fat percentage between 18 and 30%. A general health assessment including medical history, physical examination, and routine blood and urine chemistries was performed. A resting electrocardiogram was performed in order to

screen for any cardiovascular complications which would contraindicate exercise. Potential participants were excluded from the protocol if they:

- had experienced a significant amount of weight loss (>5 kg) in the last 6 months
- were habitual smokers
- were pregnant, anticipating becoming pregnant, or have experienced a loss of menses
- had habitual protein intakes of less than 0.8 g/kg body weight or above 2.0 g/kg body weight
- were unwilling to restrict their activity level to the study's requirements
- had sustained chronic musculoskeletal injuries in the past 6 months

Experimental Procedures

Peak VO_2 Determination: Each subject was tested during the screening process for peak VO_2 on a treadmill using an incremental work protocol. Briefly, after establishing a sustainable pace (i.e., in minutes per mile), the treadmill incline was increased by 2% every minute until exhaustion while VO_2 and VCO_2 were continuously monitored using a Sensor Medics Metabolic Cart (Vmax Series 29). Peak VO_2 was defined as the highest 20 second average VO_2 achieved during the last 90 seconds of the test. Attainment of peak VO_2 is generally defined by the following criteria: plateau in VO_2 with increasing workload, maximum heart rate within 10 beats of the age predicted maximum heart rate, and expiratory ratio greater than 1.10. PeakVO_2 was used to evaluate fitness level in selection of subjects for the experimental and the control groups.

To calculate average energy expenditure during running, after the subjects had a chance to recover from the peak VO_2 test, they ran on the treadmill at a self-determined "comfortable" pace for 1 mile. Near the end of the run, expired air was collected into a Douglas Bag for 1 minute for a determination of oxygen consumption rate (Zachwieja et al., 1993). Energy expenditure per mile (EEpM) was calculated using this oxygen consumption rate value. EEpM was used to determine the duration of treadmill exercise during the baseline and caloric restriction periods.

Energy Intake: Subjects reported at assigned times to the MK for breakfast and dinner. These meals were eaten under supervision at the Pennington Center. Lunch was provided in box form to be consumed off campus. Subjects were instructed to consume all and only the food provided. A snack was provided for the subject to consume at home that evening. Energy intake was adjusted to each subject's individual energy needs as determined by BMR (Harris-Benedict equation) and from the 3 day dietary record obtained during screening. During the baseline period, adjustments were made to account for the 500 kcal/day exercise energy expenditure and the protein content of the diet was 1.3 g/kg body weight.

The diet was designed to include standard foods consumed in a regular American diet, meeting the nutritional recommendations of the US Recommended Dietary Allowances; one standard menu cycle was designed for all study participants. Subjects were interviewed for

individual food allergies or intolerances, and food substitutions were made if necessary. Macronutrient composition was similar to that of garrison feeding during the baseline period for all participants and during the exercise period for controls, providing approximately 51% CHO, 14% protein, and 35% fat. During the calorie deficit phase, macronutrient composition was adjusted to reflect field consumption of 48% CHO, 15.5% protein, and 36.5% fat by maintaining high protein and fat intake composition and lowering CHO composition.

Energy Expenditure: Each day during the experiment subjects reported to the ETF to perform an acute bout of treadmill exercise. The subjects were instructed to complete the predetermined amount of exercise in the testing facility and to abstain from any physical activity outside of the ETF during this period. Resting heart rate and blood pressure were recorded prior to each session. The acute bout of exercise consisted of running (R) on a treadmill (Life Fitness, 9500HR Series) at a set pace for a time that elicited a 500 kcal energy expenditure. Heart rate and oxygen consumption (via the Douglas bag technique) were monitored during each training session.

Body Composition: Total body fat and lean body mass were determined by DEXA (Hologic QDR 2000) in the morning on day 7 of the baseline dietary period and on day 21 of the experiment. Body mass index (from body weight and height) and waist-to-hip ratios were determined during screening.

Muscle Strength Test: On day 6 of the baseline period and on day 20 of the experimental period, a one repetition maximum (1 RM) protocol was used (on BodyMasters™ resistance exercise equipment) to determine upper body (UB) and lower body (LB) strength. The Shoulder Press machine was used to determine UB strength and the Super Leg Press was used to determine LB strength. Standardized instructions explaining each test and a demonstration of proper technique was given to each subject prior to testing. After a standardized warm-up of 15 repetitions at a manageable resistance, a weight close to, but under the subject's expected maximum lifting capacity was selected. If one repetition was completed, 5 kg (10 lbs) increments were added to the exercise device until 1 RM was achieved.

Muscle Endurance Test: On day 5 of the baseline period and day 19 of the experimental period a lower body muscular endurance test was completed. This test consisted of squatting exercise with a 100 lb weight for men and a 50 lb weight for women. The subjects performed 25 repetitions per minute, timed with a metronome, and continued till exhaustion. Exhaustion was defined as the point at which the subjects could no longer keep up repetitions with the metronome. Total time for the test was recorded.

Anaerobic Capacity Test: Proceeding the muscle strength test on baseline day 6 and experimental day 20, a Wingate anaerobic capacity test (Bar-Or, 1987) was performed. The test consisted of an all out 30 second maximal effort on a variable load Cybex™ cycle ergometer at a workload equal to 0.075 kg/kg body weight. Peak power was defined as the highest power output

achieved in the first 5 seconds of a test, while mean power was defined as the average power output over the entire 30 seconds of a test.

Endurance Performance Test: In place of the treadmill exercise session on day 7 of the baseline period and on day 21 of the experiment, subjects performed a timed 5 mile running test designed to measure endurance performance. Subjects reported to the indoor Track at LSU Student Recreation Center at assigned times. Instructions and information such as number of laps per mile, and proper lane usage followed a warm-up consisting of a 1 lap jog and standardized hamstring, quadriceps, groin and calf muscle stretches. Endurance performance was defined as each individual's 5 mile time.

Urinary Nitrogen Balance: On days 6 and 7 of the baseline period and on days 20 and 21 of the experiment, subjects collected all urine excreted into individual bottles and recorded the time of each void on the bottle. The subjects were required to produce a urine void at 7am on each of the collection days. The urine specimens were divided into two 24hr periods, the total volume measured, recorded and an aliquot saved for analysis of total nitrogen content and creatinine by the Pennington Center's Clinical Laboratory.

Results/Progress

We received approval from the Army IRB on January 16th, 1998 to conduct this study. Subjects for this study were sought from the general student bodies of Louisiana State University, Southern University, and the Campus ROTC programs. Between January 26th and February 20th we recruited, screened, and enrolled 6 volunteers (3 men and 3 women) into the study. We anticipate studying 8 men and 8 women in the experimental group, and 4 men and 4 women in the control group. Thus, subsequent cohorts are planned (and/or are being conducted) to meet subject requirements. Cohorts of six are manageable not only from the standpoint of the Metabolic Kitchen's ability to feed subjects within the context of other ongoing research protocols, but also from the standpoint of supervised daily exercise training and pre- and post-training performance testing. The first six subjects enrolled into the study participated from February 25th to March 20th 1998.

All volunteers were physically active and determined to be in good health as indicated by blood and urine analysis, resting blood pressure and EKG, and physical examination. The characteristics of the 6 individuals studied are presented in Table 1 (see Appendix). The VO_2 max results from the maximal treadmill exercise test indicate that the volunteers were physically fit. During the experimental period, all meals were provided by the PBRC Metabolic Kitchen. The volunteers ate breakfast and dinner at the Center and took out lunch to eat on their own time when convenient. Body weight was recorded daily prior to eating breakfast. A Research Associate in the Metabolic Kitchen kept the daily weight records and ensured that all food provided was eaten. Daily body weight records were not disclosed to any other investigator involved with this project. Daily exercise during the experimental period was performed at the PBRC Exercise Testing Facility under supervision by one of the study investigators. Daily

exercise consisted of treadmill running at an intensity and duration sufficient to elicit around a 500 kcal energy expenditure. These exercise bouts were approximately 30-50 minutes in duration, and elevated heart rate to around 150-170 beats per minute. Oxygen consumption was measured during each day's exercise session by collecting expired air into a Douglas bag for a 1 minute period. This collection was made half-way through an exercise session. Oxygen consumption rate was converted to an energy expenditure rate (kcal/min) and then multiplied by the total exercise time to yield total energy expenditure for the exercise session. Average daily exercise energy expenditure remained stable across weeks 1 (baseline period), 2, and 3 in both men and women. Average daily exercise energy expenditure for each week of the experiment is given in the table below. One of the female volunteers had to be withdrawn from the study at the beginning of experimental week 3 do to a sprained ankle; therefore, no exercise energy expenditure data are available for female subject #3 in the third week (see Appendix, Table 2).

As we will remain blinded to group assignments until the end of the entire project (i.e., 3 more cohorts of 6), only the baseline data from the physical performance tests are presented in Table 3 and Table 4 (see Appendix).

C. Conclusions

To date, it appears that we have implemented a feasible and well controlled protocol to determine how moderate (750 kcal/day) caloric restriction in combination with daily exercise training (500 kcal energy expenditure) effects body composition, muscular strength, aerobic (endurance) performance, anaerobic capacity, and body protein balance in young fit men and women. One of the few problems we have had is complaints of muscle soreness and fatigue near the end of the first week of training. This is likely the result of an increase in the frequency (i.e., daily exercise training) of exercise in our participating subjects. Unfortunately, near the end of the first week is when we are making many of our measurements of baseline performance. We chose this time to make these measurements because we wanted all of the subjects to be participating in a similar level of training when such measurements were made. Nonetheless, we may not be making a measurement of "true" baseline performance because of the existing level of fatigue. In order to better interpret the end result from our current protocol design, we may have to conduct another experiment in which we measure performance variables before and 7 days after increased frequency (daily) in exercise training.

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V. Nutrient Database Integration Laboratory

A. Introduction

Timely receipt of dietary data via computerized nutrient analysis of recipes, menus, and dietary intakes of the soldiers is critical to assessment of the soldiers' needs and interrelationships with other aspects of military life. In addition to including within this project the analysis of recipes developed for Army garrison feeding situations, we proposed to diversify our tasks and more fully integrate with tasks of the Military Nutrition Division by providing analysis of dietary intakes taken during military feeding studies. We proposed to assist

effectively in dietary collection protocols and analysis of those collections. Our position is to advise the principal investigators on dietary collection and methods to assure a quick turnaround of nutrition information needed for statistical analyses. We work with the current nutrition staff, data programmers, and other key personnel in the development of a more effective database, somewhat patterned after the now defunct Computerized Analysis of Nutrients (CAN) system. Inclusion of the users of the dataset is essential in developing and perfecting a more efficient system that can generate finalized data in a more timely fashion than existed previously in the Military Nutrition Division.

We proposed this task based on the position that we can offer programming expertise, personnel stability, and personnel who can be involved in precise data collection, both in the garrison and field settings. Our plans included state of the art data programming and high technology data transfer and analysis as a part of our proposal. We plan to take advantage of a client-server situation which will involve data collections transferred to a central site (PBRC) for analysis. We eventually plan to integrate all Armed Forces Recipes, special formulations and feedings into one database system that can meet Military Nutrition needs for both now and the distant future, a database that will be ever changing and modifiable to the unique set of circumstances of each study or at USARIEM, in which computerized nutrient analysis of dietary intakes is undertaken. We also proposed that authorship on all future publications include at least one PBRC investigator from this task who has contributed substantially to the conduct of the study.

B. Body

Dietary information is collected during field nutrition studies with the software application MiDAS (acronym for Military Dietary Analysis System). MiDAS was developed at PBRC Nutrient Data Systems Laboratory. It was programmed in Visual Basic 5.0 (Microsoft Corporation, 1997) and utilizes a Microsoft Access Version 7.0 database to store collected data (Microsoft Corporation, 1997). Collected data is analyzed using one of two USDA datasets. Some information is derived from USDA database for standard reference, commonly referred to as Standard Release 11 (USDA, 1996). The Standard Release 12 was released in March of 1998 (USDA, 1998) and has been used to update the system. However, the studies conducted this year used the earlier release. Primarily used during field studies employing food records reflecting intakes of non-military rations is the USDA Survey Database for the Continuing Survey of Intakes by Individuals (CSFII). The version used on the studies conducted prior to 1998 was CSFII "94 (USDA, 1995). The CSFII '96 data was released in March of 1998 and has been used to update the current version of MiDAS (USDA, 1998). In addition, several studies contained military rations. When military rations are used, we contact USARIEM and/or Natick Labs for the nutritional information if we are to analyze the dietary intake information. The development of the MiDAS system was presented at the Experimental Biology meeting in San Francisco in April 1998 (Allen et al., 1998).

We processed several studies during this reporting year and revised data from a summer 1996 study which was not cited in the 1997 reporting year since the data had a number of edits to be completed. Therefore, the studies from Summer 1996 through this year will be reported, with data given which has been disseminated to the principal investigator. Military field studies may use one or more means to collect dietary intake data. During our studies, three main methods of collecting dietary intake have been used. The visual estimation method uses trained data collectors to visually estimate the quantity of food before and after the meal by comparing the portion to weighed standard portions of the same food. This method has been reported elsewhere (Rose et al., 1987). Some studies employ dietary records self-reported by the subject. In these cases, the subjects generally undergo training to some extent and the records are reviewed by data collection personnel trained to probe for additional information when reviewing the record. Several studies this past year have combined a self-reported diet record with acceptability data using a 9 point hedonic scale, routinely used by the military. The MiDAS system allows for the collection of both visual estimation data and dietary record data or a combination of more than one type of collection within the same data entry system.

Nutritional Assessment of U.S. Army Rangers in Garrison and During a Field Training Exercise in a Hot Environment. Hunter Army Airfield, GA from 13 July - 5 August, 1996

Although conducted in 1996, the data from this study has not previously included in an annual report. The data from this study was provided to the Principal Investigator, LTC John Warber, Dr.P.H., R.D., in March 1997. We have revised the data as needed and shipped the raw data forms to LTC Warber in December 1997. Those individuals taking part in this study from PBRC included Catherine Champagne, PhD, RD; Alice Hunt, PhD, RD (under subcontract with Louisiana Tech University, Ruston, LA); Ray Allen, PhD; Mary Baldwin Sanders, MS, RD; Barbara Eberhardt, BS; Anyce Griffon, BS; Philippe Hebert; Stacy Heilman; and Leslie Favie. The protocol included six days of intake in garrison gathered by visual estimation combined with food records for snacks or meals consumed elsewhere, seven days of MRE and pogey bait intake during the field training exercise using special food record forms developed by Natick, and an additional two days of intake recorded by food records designed to determine types of food choices following the hot environment field training.

PBRC processed the garrison intakes and the intakes following the field training. The total number of lines of data entered was 7,531. PBRC was responsible for the data entry for the field exercise portion of the study which involved MRE and pogey bait collection, however the data analysis for that portion was done by Natick Labs. The following tables and figures resulted from the portion of data collected and processed by PBRC:

Conclusions on the Nutritional Quality of the Ranger Diet in Garrison

- Reported mean food energy intakes were slightly below recommended energy intakes for moderately active adults (3000-3200 kcal).
- Mean intakes of most vitamin and minerals met the MRDA recommended values for gender. mean intake of B6 was low, 2.4mg (2.8mg)

mean intake of magnesium was low, 325mg (350-400mg).

- In general, Rangers subsisting in garrison dining facility were following the principles of the 1995 Dietary Guidelines for Americans.
  mean percentage of total energy from fat was 30.7%, recommended 30%
  mean percentage of total energy from saturated fat was 10.7%, recommended 10%
  mean intake of dietary cholesterol was 349mg, recommended less than 300mg

Data from the Savannah Rangers Study was presented as a poster session at the 16th International Congress of Nutrition in Montreal, Canada in July 1997 (Champagne et al., 1997)

Nutritional Assessment and Dietary Education of U.S. Army Sergeants Major Academy Students in Garrison. A Three Phase Study conducted in September, 1996; December, 1996, and March 1997. Biggs Army Airfield, Fort Bliss, El Paso, TX

This study was initiated during the 1996-97 reporting year, however has not previously been referred to in the annual report. The data was provided to the Principal Investigator, MAJ William H. Karge, III, Ph.D., in July 1997. We have revised the data as needed and shipped the raw data forms to MAJ Karge in December 1997. Those individuals taking part in this study from PBRC included Catherine Champagne, PhD, RD; Ray Allen, PhD; Barbara Eberhardt, BS, RD; Anyce Griffon, BS; April Hebert, BS, RD; Philippe Hebert; Fatemah Ramezanzadeh, MS; Regina Louviere, BS; and dietetic interns from Louisiana Tech University, Ruston, LA and North Oaks Hospital, Hammond, LA.

The protocol included three days of intake recorded by the subject and reviewed by the data collector. Intakes were Sunday, Monday, and Tuesday intakes with interviews with the soldiers scheduled on Monday, Tuesday and Wednesday to review the previous day's intake.

PBRC processed the dietary intake data. The total number of lines of data entered was 12,498.

Objectives:

- Evaluate the nutritional adequacy of diets of career NCOs
- Assess changes in diet after health promotion courses
- Assess changes in cholesterol levels in entire class

Study Design

- PBRC and USARIEM investigators used dietary analysis to assist in measuring the effectiveness of dietary education in the most recent class
- PURPOSE
  To decrease serum cholesterol levels and coronary heart disease risk in soldiers
- SAMPLE SIZE - 106 Soldiers
- STUDY DESIGN
  Blood draw and diet records pre, after 10 weeks, after 20 weeks

Dietary education at beginning of program

- DEPENDENT MEASURES
  Serum lipids: Total Cholesterol, LDL, HDL, Triglycerides
  Body composition: weight, height, body fat
  Dietary analysis

Data from the El Paso Study was presented at the Experimental Biology meeting in San Francisco in April 1998 (Champagne et al., 1998).

Assessment of Nutritional Status and Energy Expenditure and Determination of Gender Differences in Dietary Intakes of Combat Service Support Personnel Subsisting on Meal-Focused Versions of the Meal, Ready to Eat, Camp Mackall, North Carolina. 29 April 1997 to 13 May 1997

This study involved collection of MRE intake data. Data entry was on site and involved the largest number of people to date to input data into the MiDAS system. PBRC was responsible for the data entry only, and the data files were given to the Principal Investigator, Cory Baker-Fulco, before departure from Camp Mackall. The following individuals from PBRC assisted in this study: Catherine Champagne, PhD, RD; Ray Allen, PhD; Bill Glover, PhD; Barbara Eberhardt, BS, RD; April Hebert, BS; Troy Fontenot, BS; Eric LeBlanc, BS; Kelly Patrick, BS; Fatemeh Ramezanzadeh, MS; Regina Louviere, BS; and Alan Pesch, BS. The following information was derived from both the proposal and some of the data collection on acceptability, and was presented at the PBRC Work In Progress seminar series in May 1997, just following our return from the study.

Objectives:

- To determine gender specific differences in MRE component selection and preferences.
- To assess nutrient intakes of Army combat service support personnel subsisting on two different MRE rations (version XVII and a meal focused, carbohydrate-enhanced version.
- To estimate average energy expenditures of male and female combat service support personnel during a 14-day field training exercise.
- To ascertain the knowledge and attitudes of male and female soldiers toward MREs.
- To ascertain the dietary habits of male and female combat service support personnel when in garrison.

Data Collection:

- Food and fluid intake
  MRE cards for lunch only for 5 days plus pogey bait
  MRE cards for 3 meals plus pogey bait for 7 days
  Fluids measured in canteens, canteen cups, and beverage cups
- Food preference and acceptance using 9-point hedonic scale
- Typical practices and nutrition knowledge
  Diet Habit Survey (Conner, Oregon)

Nutrition Knowledge and Attitudes

- Blood analyses
- Body height, weight, and anthropometric data
- Questionnaires
  - Demographic
  - Final Ration Opinion Questionnaire
- Energy expenditure, total body water and water turnover on a subsample of 36 volunteers to include 23 women
- Activity monitors on a subset of the DLW volunteers
- Foot contact monitors to estimate the metabolic cost of walking and running based on body weight and the time during each stride that a single foot contacts the ground
- Subjects and Data Collection
- There was data collected on 263 total subjects
  - 169 males (64.3%)
  - 94 females (35.7%)
- A total of 4061 meal cards were processed
  - MRE consumption, rating, reason not finished
  - beverage consumption (bulk)
  - pogey bait
- A total of 53,131 lines of data was entered

The remainder of the slides presented at the Work In Progress Seminar Series are included in the Appendix.

Impact of Creatine Intake on Physical Performance, Fort Bragg, North Carolina. 28 August 1997 through 28 September 1997

In this study data was collected during three phases during August and September 1997. The data was provided to the Principal Investigator, LTC John Warber, Dr.P.H., R.D., in December 1997. Those individuals taking part in this study from PBRC included Catherine Champagne, PhD, RD; Ray Allen, PhD; Barbara Eberhardt, BS, RD; and April Hebert, BS, RD.

Twenty-seven Rangers participated in the study and three-day dietary intake records were processed at three different times during the study: baseline, following one week of placebo, and following one week treatment with creatine. The intake portion of the study began on September 5 and was completed on September 24, 1997. The protocol included three days of intake recorded by the subject and reviewed by the data collector during each of the three phases of the study. Intakes were Thursday, Friday, and Saturday intakes with interviews with the soldiers scheduled on either Monday or Tuesday, as their schedule permitted, to review the data.

PBRC processed the dietary intake data. The total number of lines of data entered was 3,857. Some preliminary data was released to LTC Warber, therefore we will include final data in our next annual report.

The Effects of Carbohydrate Supplementation on the Performance of Combat Relevant Activities. Fort Lewis, WA, February 8-13, 1998

The following personnel from PBRC assisted in the USARIEM study on the ERGO carbohydrate beverage which was completed under the direction of MAJ Stephen Slade, the British Exchange Officer stationed at Natick.: Catherine Champagne, PhD, RD; Ray Allen, PhD; Eric LeBlanc, BS; Barbara Eberhardt, BS, RD; Bradley Prather, BS, RD; and Alana Cline, PhD, RD. This protocol involved a field test to identify whether the Ergo Drink improves physical performance in typical military scenarios. The study was designed to identify whether the consumption of the ERGO (Energy Rich Glucose Optimised) Drink developed by Natick RD&E Center can help to improve "performance" while performing typical, physically arduous military tasks in the field.

PBRC processed the dietary intake data. The total number of lines of data entered was 5,080. Preliminary data from the study was presented to MAJ Slade on March 6, 1998, during a visit to USARIEM. A more finalized dataset for this study will be released in June 1998.

C. Conclusions

During this year, several studies have come to complete closure in terms of dissemination of nutritional intake information, namely the Savannah and El Paso studies. The Camp Mackall study indicated our ability to enter dietary data on an extensive number of subjects and provided delivery of that data on the final day of the study. For the Camp Mackall study, we had been advised that of a planned 300+ subjects, data was to be entered on about half of the subjects. This did not turn out to be the case, however, and all data on all subjects was entered for an almost unimaginable 50,000+ lines of data (we had approximately 5 data entry people working 8-12 hours each day).

We believe that we are currently carrying out the mission of this task, efficiently and effectively working to deliver nutritional intake information from USARIEM's field studies that we are involved in. We are working to further streamline delivery of dietary intake information and setting up a version of the MiDAS system which can be used from the USARIEM end.

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VI. Enhancing Military Diets

A. Introduction

The current task is an expansion of Task 5: Menu Modification Project, which involved development and incorporation of recipes modified in fat, sodium, and cholesterol into the garrison menu to lower percentage of energy consumed from fat by young soldiers. Recipes were developed that incorporated ethnic preferences as well as vegetarian and breakfast items; they were successfully validated at Louisiana Tech University and Ft. Polk, LA. Modified recipes were introduced into the menu at Fort Polk as one component of a cardiovascular risk assessment study that compared nutritional intake of soldiers selecting from the regular menu versus the modified menu. Passive intervention methods were utilized; the soldiers were not aware that they were being offered modified-fat foods. Modified recipes successfully lowered mean fat intake from 34% to 31% of energy. Of concern, however, is the continued consumption of high-fat foods away from the dining facility.

The purpose of this project is to incorporate additional modified recipes into the Armed Forces Recipe File, instruct staff and students at the Army Culinary Arts School on selection and preparation of lower fat foods, assess dining hall consumption of menu items, and implement a nutrition education component.

B. Body

Taste panel procedures were modified to provide a more formal rating process, incorporating the hedonic evaluation used for military rations for almost 40 years (Peryam & Pilgrim, 1957). To better evaluate sensory attributes of recipes being tested in the consumer taste test panel, a new scannable evaluation form was developed, the format more closely following evaluations being used at Natick Labs in their consumer panels. A copy of the evaluation form can be found in the Appendix. Data are analyzed and incorporated into a computer program developed by Dr. Ray Allen at PBRC to consolidate all phases of development and testing of recipes as they are formulated. Automation of the forms now allows breakdown of ratings by category: age, gender, and military experience, enabling us to determine whether recipes are acceptable to our target audience of the younger service member.

A food preference survey was developed for administration at selected military installations to determine current food trends and selections in the young military population. We used as a guide the survey conducted by Natick labs in 1992-93 (Kluter et al., 1994). Our survey was designed to target more specifically questions addressing fat intake and preferences of foods with varying fat content, questions which were not included in the previous study. We will use results from our survey to develop recipes acceptable to the target population. A food preparation survey was also developed for military cooks to evaluate types of food preparation equipment and techniques they most frequently use for meal preparation. In response to requests by the Armed Forces Recipe Service, we have expanded our survey administration to members of all military services. Copies of the survey questionnaires can be found in the Appendix.

In quarterly consumer taste panels, 38 recipes were developed and/or modified and tested. From those recipes, 10 met all nutrient and acceptability standards at PBRC, were validated at Louisiana Tech, and will be forwarded to the Armed Forces Recipe Service for approval and incorporation into the recipe file. The remaining recipes are in the development and revision stage and will be re-evaluated in future taste tests; we will continue to test 10-12 recipes per quarter.

Food preference surveys were distributed in dining facilities at 3 military installations to 690 individuals; 662 were returned (95.9%). Gender distribution was 61.8% male, 38.2% female; 64% were in the 18-21 y.o. range. All areas of the country were represented in geographic distribution of respondents (see Appendix, Table 1). Major findings include the following: more frequent selection of "low-calorie/diet food" by women (33% vs 19% daily); more frequent selection of "power/energy food" by men (33% vs 23%); no differences in

selection of "fast food/convenience food" (men - 12%, women - 10% daily), or "heart healthy/low cholesterol food" (men - 34%, women - 35%) (see Appendix, Table 2).

Gender differences in food preference include: vegetarian (women - 5.8 rating, men - 4.6 rating); women preferred chicken to beef and pork, men gave higher ratings for spicy foods, women gave higher ratings to non-egg breakfast items, low acceptability of baked and broiled fish by both genders; top ethnic food choices were Italian (men - 7.3 rating, women - 7.1 rating), Mexican (men - 6.7 rating, women - 7.0 rating), and Chinese (men - 6.4 rating, women - 6.7 rating).

Comparisons between hedonic ratings of foods by consumer taste panels at PBRC and LA Tech are shown in Table 3 (see Appendix). Average attendance of evaluators at PBRC was 73/day, at LA Tech 66/day; LA Tech had a higher percentage of evaluators in the 18-25 y.o. category (52% vs PBRC 36%); gender breakdown was the same at both sites with 36% male and 64% female at LA Tech, 35% male and 65% female at PBRC. As has been reported in previous comparisons, differences exist between sites in hedonic ratings. This is most likely due to differences in composition of consumer panels between sites (LA Tech has higher percentage of young respondents) and differences in food preparation experience of students at LA Tech, which would more accurately reflect experience of military cooks at various installations. All recipes evaluated, however, met the acceptability and nutrient analysis criteria for inclusion in the Armed Forces Recipe File and are being forwarded for approval by the AFRS committee.

Members of the DOD Food Policy Council and Armed Forces Recipe Service held their meeting at PBRC November 1997 to discuss progress of recipe development and coordinate dissemination of recipe changes among the services. PBRC will maintain ongoing communication with Pam Beward, committee chair, to report on current progress. We will provide quarterly information on recipes being developed or evaluated.

Our research team traveled to the Army Culinary Arts School at Ft. Lee, VA to coordinate plans for a "train the trainer" workshop on low-fat cooking and a future consumption study in the dining facilities. The staff was very receptive and willing to work with us but were undergoing a temporary staffing shortage. Plans are to arrange both the training and study in Fall 1998 or Spring 1999 when staffing at the school is expected to increase. The Navy Culinary School at Norfolk Naval Base has also expressed an interest in a similar workshop.

C. Conclusions

Approximately one-third of young service members report they are currently consuming heart healthy foods daily, but one-third also report consuming fast foods 3-4 times per week, indicating a need for nutrition education and additional availability of lower-fat foods for this population. The currently developed recipes have received favorable ratings by consumer panels unaware that fat modification techniques were used in the individual foods evaluated. It will be

important to compare these ratings with a population that is aware of the modifications to determine what impact nutrition knowledge has on perceived food acceptability.

D. References

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VII. Stress, Nutrition and Immune Function Laboratory

A. Introduction

Participation in physically rigorous military training results in both physical and mental stress (1). Some of the stressors associated with training include caloric deficiencies, sleep deprivation and exercise. Concomitant with these stresses is an alteration of a number of parameters of lymphocyte-related immunity (2-4). Though the significance of these *in vitro* alterations in immune responses is unclear (4), an increase in documented infection rates coincided with indications of compromised immune function in US Army Rangers course participants (5). Thus the characterization of the mechanism of stress-induced alterations in immune responses could lead to the development of preventative strategies for reducing this immunomodulatory effect and improving the health of the soldiers. While the precise mechanism of this altered immune responses remains unknown, it is likely that inadequate energy intake contributed to this phenomena (3).

The nutritional status of an individual can be a very important component of the stress response, either being directly responsible for the stress (6, 7) or affecting the response to other stressors (8, 9). While moderate dietary energy restriction prolongs life-span and enhances immune responsiveness (10), protein-energy malnutrition is associated with decreased lymphocyte proliferation reduced cytokine release, and lower antibody response to vaccines (7) and enhancement of tumor metastasis (11). On the other hand, excessive intake of nutrients also impairs immunity (12). Polyunsaturated fatty acids (PUFAs) are structurally important for cell membranes and play a significant role as precursors (esp. arachidonic acid) of eicosanoids (prostaglandins, thromboxanes, leukotrienes). However, these eicosanoids may produce exaggerated effects in acute stress responses causing immunosuppression (8). The rate of eicosanoid synthesis is determined by PUFA turnover and this is enhanced by stressful conditions such as trauma and sepsis (13). The nature of eicosanoids produced is determined by the availability of different PUFAs in the cellular phospholipid pool. Omega-6 and omega-3 PUFAs give rise to different eicosanoids (13) with the eicosanoids formed from omega-3 PUFAs seeming to be more beneficial to the immune system compared to those derived from omega-6

PUFAs (14). This has led to the suggestion that the amount and type of dietary fatty acids can influence *in vitro* measures of immune function (15) and that dietary composition may need to be altered during stressful conditions (16).

In order to determine the effect of diet on stress-induced immune modulation, we have developed two models of stress-induced immune modulation in collaboration with Dr. Ruth Harris. Her work, detailed elsewhere in this report, has demonstrated that rats subjected to either a restraint or sleep-deprivation, exhibit profound evidence of physiologic stress, including decreased food consumption and an overall loss in body weight (see also Figure 1 in Appendix). Thus in several ways this stress model mimics some of the changes observed in the Army Rangers course (3). During this past year our efforts have focused on characterizing the effect of these stresses on immune function in the rats. Immune function was determined both using *in vitro* and *in vivo* measures.

B. Body

Materials and Methods

Animals. Male Sprague-Dawley rats (600 g) were used in all experiments. Rats were caged individually with *ad libitum* access to food and water.

Sleep Deprivation. Rats were sleep deprived by placing them into pedestal-style cages in which a small platform is surrounded by water. Rats are allowed free access to food and water while on the platform but they cannot lie down. Rats were subjected to sleep deprivation for 24 - 72 hours.

Restraint. Rats were restrained in ventilated plastic tubes. Each rat was restrained for four hours a day for four consecutive days.

Diet. Rats were fed either a low fat (5% kcal from fat) or high fat (45% kcal from fat) diet as a pelleted ration. The composition of the fat in the diets was 14% saturated, 29% monounsaturated and 57% polyunsaturated. Rats were placed onto the test diet one week prior to stressing and remained on the diet throughout the stress period.

Vaccination. Vaccinated rats received a single intramuscular injection of 100 ÿg of maltose binding protein (MBP) with alum adjuvant. Rats were injected immediately prior to stressing. Control rats were injected with the same vaccine preparation at the same time as the stressed rats.

ELISA. Antibodies to MBP were determined by ELISA. Plates were coated with MBP (5 ÿg/well) and blocked with 1% fish gelatin. Serum samples were serially diluted and added to the plate in duplicates. Rabbit anti-rat-Ig conjugated with horse radish peroxidase was used to detect the rat antibodies. Plates were developed with the TMB substrate (KPL Laboratories) and read on a microplate reader.

Lymphocyte proliferation. Splenic lymphocytes (2 x 10^5) were incubated for three days with mitogens (pokeweed (PWM), phytohemagglutinin (PHA) and concanavalin A (ConA) or five days with MBP (0.5 ÿg) at 37° C in a CO_2 incubator. Plates were pulsed for 4 hours with 0.5 ÿCi of ^{3}H-thymidine and the DNA harvested onto filter pads for liquid scintillation counting. Stimulation indices (SI) were calculated as CPM of stimulated cultures/ CPM of medium controls. All determinations were performed in triplicate.

IL-2 receptor expression. The expression of the high affinity receptor for IL-2 (IL-2R) was determined using flourescently conjugated recombinant human IL-2 and a flow cytometer. Cells were incubated for 72 hrs with PHA and then washed with PBS. Recombinant human IL-2 conjugated with phycoerythrin was added to the cells. After washing with ice-cold PBS, the cells were fixed with 2% paraformaldehyde and then analyzed for fluorescence using a FACScan. The percentage of IL-2R positive cells was determined using pre-determined cutoffs for positive and negative staining cells.

Results

Preliminary experiments have been conducted regarding the effect of sleep deprivation on the immune response of rats. Similar to results reported by Dr. Ruth Harris, rats subjected to sleep deprivation exhibited losses of body weight initially following sleep deprivation (see Appendix, Figure 1), but were able to recover these losses by 30 days after the stress. Initial studies demonstrated that this stressor produced significant inhibition of the *in vitro* lymphoproliferative responses to various mitogens (see Appendix, Figure 2) with the degree of inhibition being dependent upon the duration of the sleep deprivation.

The cytokine interleukin 2 (IL-2) plays a central role in regulating lymphoproliferative responses. In order to determine if the observed immune modulation was the result of the altered expression of this cytokine, recombinant human IL-2 (60 u/ml) was added to the cultures. While the addition of the cytokine increased the overall proliferative response in the control cultures, it failed to overcome the stress-induced suppression (see Appendix, Figure 3).

In addition to analyzing the *in vitro* effect of sleep deprivation stress on immune function in the rats, we have also begun to determine the effect of this stressor on the generation of an immune response *in vivo*. Rats were immunized with the protein antigen, maltose binding protein (MBP), prior to sleep deprivation. Both humoral (antibody) and cellular (lymphoproliferative) immune responses were then assessed one month later. Sleep deprivation for both 24 and 72 hours had a significant effect on the ability of these rats to develop antibodies to the protein vaccine (see Appendix, Figure 4). Similar results were obtained with the lymphoproliferative response, though the overall level of proliferation was quite low (data not shown), probably the result of using alum as an adjuvant for the vaccination. While alum does serve to augment the antibody response to protein antigens, it only weakly simulates a T cell

response. Future experiments will include the use of other adjuvants in hopes of better stimulating the T cell component.

In order to assess the effect of diet on stress-induced immune modulation, two groups of rats were fed either a low fat or high fat diet and subjected to four consecutive days of restraint. The splenocytes of those restrained rats fed the high fat diet exhibited significant suppression of the proliferative response to PWM (see Appendix, Figure 5). Similar results were obtained with PHA and ConA (data not shown). The addition of recombinant IL-2 (60 u/ml) failed to overcome this suppression of the proliferative response in the cultures from the high fat fed, restrained rats (see Appendix, Figure 6). Subsequent analysis of IL-2 receptor (IL-2R) expression revealed that cells from restrained rats exhibited higher levels of IL-2R expression than those of unrestrained controls, even though their proliferative response was reduced.

Discussion

While it is apparent that participation in physically rigorous military training results in alteration of a number of parameters of lymphocyte-related immunity (2-4) which may result in an increased incidence of disease (5), the etiology of this immune modulation remains unclear. It is generally supposed that the multiple stressors associated with this training may be responsible for these effects (2-5). There have been numerous reports indicating that physical, chemical or psychosocial stress can result in immune modulation and an increased susceptibility to disease (for review see (17)). The adverse effect of stress on health and well-being is widely recognized (18). These and other studies have led to the concept that there are interactions between the immune system and the central nervous system and that the immune system may be modified by stress to produce inappropriate changes in immunocompetence (19). The demonstration of soluble mediators and receptors common to these two systems further supports this notion (20). Little information is available, however, regarding the specific interactions which may be involved in stress-induced immune modulation. The reasons for this uncertainty include the complexity of the stress and immune responses (21).

A number of model systems have been reported for studying stress-induced changes in immune function (22). Results from these studies have demonstrated that few generalizations are possible because the direction and/or magnitude of the effects of stress in modulating immune responses clearly depend on the stressor, the nature of the immune response and the immune compartment in which it is measured, the time of sampling, a variety of host factors (e.g. species, strain, age, sex), and interactions among these several variables (21).

The possible contribution of sleep deprivation in the immune modulation associated with physically rigorous military training has been suggested (2-4). The adverse effect of sleep deprivation on immune function is likewise widely recognized (23). Studies in both humans and animal models have demonstrated multiple effects of sleep deprivation on immune function (24, 25). In our hands, sleep deprivation of the rat results in profound effects on both *in vitro* and *in vivo* immune responses. We observed a time-dependent effect of sleep deprivation on the ability

of rat splenocytes to proliferate in response to the mitogens PHA, ConA and PWM. Our results are thus consistent with other reports of sleep deprivation inhibition of lymphoproliferative responses (24, 25) as well as other stress models in the rat (26). We also determined that the addition of IL-2 to these cultures failed to overcome this inhibition. These novel results suggest that sleep deprivation probably inhibits lymphocyte proliferation at a step following IL-2 binding to its receptor and provide important clues as to the mechanism of sleep deprivation-induced immune modulation. We also demonstrated that sleep-deprived rats responded poorly to a vaccine. These results are highly significant since they associate sleep deprivation with altered immune responses *in vivo*. While others have suggested that sleep deprivation leads to increased susceptibility to disease (23), the precise mechanism remains unclear. By using a vaccine model we may be able to address some of these issues. Furthermore, the results from these experiments may be of particular significance to the military since vaccination is an important component of the medical preparedness of military personnel.

We have utilized a second well-described model for stress to assess the possible contribution of a high fat diet on this response. Restraint stress is a widely used model for inducing stress-induced changes in immune function (27-36). While generally considered a milder stressor than sleep deprivation, the intensity of the effect of restraint stress is dependent upon the duration and frequency of the stress (28, 30, 31, 36). We have utilized a regiment of 4 consecutive days of restraint based on preliminary data from Ruth Harris' laboratory indicating that this level of restraint did alter other physiologic parameters in the rats. While a modest effect of this restraint stress on immune function was observed, a much more dramatic effect was seen when the rats were fed a high fat diet prior to and during the restraint. Our reason for focusing on a high fat diet was two-fold. First, there is a considerable body of data indicating that dietary fats can influence immune responses (37-41), particularly during periods of stress (42, 43). Second, since caloric deficiencies associated with military training is thought to be a contributor to the alterations in immune function (2, 3), the incorporation of high caloric foods to combat these deficiencies may be contemplated (44). Our results indicated that a high fat diet exacerbated the stress-induced immune modulation caused by restraint. However, since our source of fat in these diets was mixed, the possible contribution of specific fatty acids could not be determined. Future studies using this model, and the sleep deprivation model, and more defined diets will address this issue.

Similar to our results with the sleep deprivation model, we were able to demonstrate that the alteration in the lymphoproliferative response of the splenocytes from the sleep deprived rats on the high fat diet was not due to failure to produce IL-2 since addition of this cytokine to the cultures failed to overcome this effect. Furthermore, flow cytometric analysis of IL-2R expression demonstrated that the stressed rats actually had a higher level of expression than the non-restrained controls. These results suggest that the mechanism of stress-induced immune modulation may involve a step following IL-2 binding to its receptor. Others have suggested that stress-related defects in T cell proliferation occur at sites other than or in addition to these early events in cellular activation (45). Future studies are planned to further identify the specific defect occurring in our model system.

C. Conclusions

Two model systems for investigating stress-induced immune modulation were developed: a restraint stress model which provides a mild-moderate stress and a sleep-deprivation model which caused a more severe stress.

Sleep deprivation resulted in a loss of body weight and diminished *in vitro* lymphoproliferative response. Sleep deprivation also interfered with the rats' ability to respond to a test vaccine. While control rats produced both humoral and cellular immune responses to the vaccine, the sleep deprived rats failed to respond. It is noteworthy that this effect was present after only 24 hours of sleep deprivation even though ***in vitro*** responsiveness to mitogen was not affected at this time.

The restraint model produced a diet-dependent change in the lymphoproliferative response of the rats. Splenocytes from rats on a low fat diet exhibited a minor alteration in their lymphoproliferative response to the mitogen following restraint. By contrast, splenocytes from restrained rats on a high fat diet exhibited a marked alteration in this response. The addition of IL-2 to these cultures failed to overcome this inhibition, even though IL-2R expression was elevated compared to unrestrained controls. These results indicate that the block in the proliferative response could be occurring post-receptor binding, perhaps involving the receptor signaling pathway for IL-2. This possibility is currently under investigation.

The possible impact of alternative fat sources on both stress models will be developed in the upcoming year. Particular emphasis will be placed on comparing the effects of omega-3 and omega-6 PUFAs on both baseline immune responses as well as on stress-induced immune modulation in the two model systems.

The results of this work will be of particular interest to the Army since it may both identify the mechanism of stress-induced immune modulation and provide a rational basis for potential therapeutic (dietary) intervention.

D. References

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VIII. Metabolic Unit Project

At the time of our last review by the Committee on Military Nutrition Research, we were advised to maintain the opportunity for an inpatient research effort under this grant. Thus, the "Metabolic Unit Project" will be carried and will be inactive unless we negotiate a specific project with our grant officer. There was no activity on this project during the first year of the grant.

APPENDIX
INTRODUCTION

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

December 16, 1997

REPLY TO
ATTENTION OF:

Animal Care & Use Division

SUBJECT: DOD Animal Use Reporting Requirements

DONNA H RYAN
LOUISIANA STATE UNIVERSITY
PENNINGTON BIOMEDICAL RESEARCH CENTER
6400 PERKINS ROAD
BATON ROUGE LA 63110

Contract No. 92V2009

Dear Principal Investigator:

Reference your grant/contract with the United States Army Medical Research & Materiel Command (USAMRMC). Congress has mandated that the Department of Defense (DOD) collect data on animal use in DOD sponsored research, and DOD regulations require that a copy of the **most recent** U.S. Department of Agriculture (USDA) Inspection Report (**APHIS Form 7008 or equivalent, with attachments**) of sponsored facilities be reviewed on an annual basis.

Please provide a copy of the following:

a. The **most recent** USDA inspection report(s) for the institution at which you conduct animal work and/or any subcontracting facilities.

b. Also complete and return the enclosed animal use report to this office. This report should contain data relevant to your grant/contract with the USAMRMC, regardless of where the work was performed.

The Chair of your Institutional Animal Care and Use Committee and/or your Attending Veterinarian should be able to provide assistance in meeting these requirements. Prompt compliance with this request will help to prevent funding delays, please submit them **immediately**. These documents are required annually and are **due on December 1** for the period covering the prior government fiscal year, October 1 through September 30. If you have already sent both of these documents please disregard this letter. However, if you have only sent one of these documents please provide the other document, or both documents as appropriate, to U.S. Army Medical Research and Materiel Command, ATTN: MCMR-RCQ-AR (**Major Ruble**), 504 Scott Street, Fort Detrick, MD 21702-5012. Documents may be FAXED to this office at 301-619-7803.

If you have any questions please contact this office at (301) 619-2144, or Email the undersigned at MAJ_DAVID_RUBLE@ftdetrck-ccmail.army.mil

Sincerely,

David L. Ruble

David L. Ruble, DVM
Major, U.S. Army
Chief, Animal Care & Use Division

Enclosure

U.S. Army Medical Research and Materiel Command Animal Use Report

Facility Name: Pennington Biomedical Research Ctr. Principal Investigator: Donna H. Ryan
(Signature)

Address: 6400 Perkins Road
Baton Rouge, LA 70808

Principal Investigator: Donna H. Ryan
(Typed/Printed Name)

Contract Number: 92V2009

This Report is for Fiscal Year (01 October - 30 September): 96-97

AAALAC* Accreditation Status (circle one): (Full) Provisional Not Accredited

Date of Last USDA Inspection: 5/13/97 USDA Registration Number: 72-R-003 SITE 4

Definitions of Column Headings on Back of Form					
A. Animal	B. Number of Animals Purchased, Bred, or Housed but not yet used	C. Number of Animals Used Involving No Pain or Distress	D. Number of Animals Used in which appropriate anesthetic, analgesic, or tranquilizing drugs were used to Alleviate Pain	E. Number of Animals Used in which Pain or Distress was not Alleviated	F. Total Number of Animals (Columns C+D+E)
Dogs					
Cats					
Guinea Pigs					
Hamsters		60			60
Rabbits					
Non-human Primates					
Sheep					
Pigs					
Goats					
Horses					
Mice		136			136
Rats			640		640
Fish					
List Others:					

*AAALAC - Association for the Assessment and Accreditation of Laboratory Animal Care

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

ANIMAL CARE INSPECTION REPORT

☒ Routine ☐ Reinspection ☐ Pre-license ☐ Attempted ☐ Other

1. LICENSE NO./REGISTRATION NO.: 72-R-003 SITE 4
2. PAGE: 1 OF 2
3. DATE OF INSPECTION: 5-13-97
4. TIME: 3:00pm
5. DATE OF LAST INSPECTION: 4-15-96
6. TIME: 2:00pm

7. NAME AND MAILING ADDRESS OF LICENSEE OR REGISTRANT
La. State Univ. System
3810 West Lakeshore Drive
Baton Rouge LA 70808

8. ADDRESS OF PREMISES AT TIME OF INSPECTION (If different than item 7)
Pennington Biomedical Researc
6400 Perkins Road
Baton Rouge LA 70808

STANDARDS AND REGULATIONS	DOGS A	CATS A	GUINEA PIGS B	HAMSTERS B	RABBITS C	PRIMATES D	MARINE MAMMALS E	OTHER F
9. NO. OF ANIMALS INSPECTED	3			9				

"X" if in compliance; "O" if Non-compliant items (explain on APHIS FORM 7100, Continuation Sheet); NA if not applicable; NS if not seen.

FACILITIES – GENERAL	10. Structure and Construction	3.1	X	3.1	3.25	3.25	X	3.50	3.75	3.101	3.125
	11. Condition and Site	3.1	X	3.1					3.75	3.101	
	12. Surfaces & Cleaning	3.1	X	3.1					3.75	3.101	
	13. Utilities/Washrooms/Storage	3.1	X	3.1	3.25	3.25	X	3.50	3.75	3.101	3.125
	14. Drainage and Waste Disposal	3.1	X	3.1	3.25	3.25	X	3.50	3.75	3.101	3.125
FACILITIES – INDOOR	15. Temperature/Ventilation/Lighting	3.2	X	3.2	3.26	3.26	X	3.51	3.76	3.102	3.126
	16. Interior Surfaces	3.2	X	3.2	3.26	3.26	X	3.51		3.101	
	17. Drainage									3.101	3.126
FACILITIES – SHELTERED	18. Temperature/Ventilation/Lighting	3.3	NA	3.3					3.77		
	19. Shelter from elements	3.3	NA	3.3					3.77		
	20. Surfaces	3.3	NA	3.3							
	21. Capacity/Perimeter fence/Barrier								3.77		
FACILITIES – OUTDOOR	22. Restrictions or Acclimation	3.4	NA	3.4	3.27	3.27	NA		3.78	3.103	
	23. Shelter from elements	3.4	NA	3.4	3.27			3.52	3.78	3.103	3.127
	24. Drainage				3.27			3.52			3.127
	25. Construction	3.4	NA	3.4	3.27				3.78	3.101	
	26. Capacity/Perimeter fence/Barrier								3.78	3.101	3.125
FACILITIES – MOBILE	27. Temperature/Ventilation/Lighting	3.5	NA	3.5					3.79		
	28. Public Barrier								3.79		
PRIMARY ENCLOSURE	29. General Requirements	3.6	X	3.6	3.28	3.28	X	3.53	3.80	3.104	3.125
	30. Space & Additional Requirements	3.6	X	3.6	3.28	3.28	X	3.53	3.80	3.104	3.128
	31. Protection from Predators	3.6	X	3.6	3.25	3.25	X	3.52	3.80	3.101	3.125
ANIMAL HEALTH AND HUSBANDRY	32. Exercise and Socialization	3.8	X								
	33. Environment Enhancement								3.81		
	34. Feeding	3.9	X	3.9	3.29	3.29	X	3.54	3.82	3.105	3.129
	35. Watering	3.10	X	3.10	3.30	3.30	X	3.55	3.83	3.106	3.130
	36. Cleaning and Sanitation	3.11	X	3.11	3.31	3.31	X	3.56	3.84	3.107	3.131
	37. Housekeeping and Pest Control	3.11	X	3.11	3.31	3.31	X	3.56	3.84	3.107	3.131
	38. Employees	3.12	X	3.12	3.32	3.32	X	3.57	3.85	3.108	3.132
	39. Social Grouping and Separation	3.7	X	3.7	3.33	3.33	X	3.58		3.109	3.133
TRANSPORTATION	40. Primary Enclosure	3.14	X	3.14	3.36	3.36	X	3.61	3.87	3.113	3.137
	41. Primary Conveyance	3.15	X	3.15	3.37	3.37	X	3.62	3.88	3.114	3.138
	42. Food and Water	3.16	NS	3.16	3.38	3.38	NS	3.63	3.89	3.115	3.139
	43. Care in Transit	3.17	NS	3.17	3.39	3.39	NS	3.64	3.90	3.116	3.140
	44. Handling during Transportation	3.19	NS	3.19	3.41	3.41	NS	3.66	3.92	3.118	3.142

45. Identification	2.38 X	2.50					
46. Records & Holding Period	2.35 X	2.38	2.75-2.78	2.101	2.132	2.133	
47. Handling	2.38 X	2.131					
48. Veterinary Care	2.33 X	2.40	3.110				
49. IACUC	2.31 X						
50. Personnel Qualifications	2.32 X						
51. Other items?	YES (If yes, see continuation sheet)		√ NO 68				

52. PREPARED BY (Signature and title)
Lynn P. Bourgeois 4002
Veterinary Medical Officer
53. DATE: 5-13-97

54. COPY RECEIVED BY (Signature and title)
Clinical Veterinarian
55. DATE: 5-13-97

56. REVIEWED BY (Signature and title)
57. DATE

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

CONTINUATION SHEET FOR ANIMAL CARE INSPECTION REPORT (S)

(APHIS FORMS 7004 and 7008)

1. LICENSEE OR REGISTRANT AND NUMBER	2. LIC. OR REG. NO.	3. DATE	4. PAGE
La. State Univ. System	72-R-003 SITE 4	5-13-97	2 OF 2

5. LOCATION OR SITE	6. WAYBILL NUMBER AND DATE *(If Applicable.)*
Pennington Biomedical Researc 6400 Perkins Road Baton Rouge LA 70803	

7. NARRATIVE: I. Non-compliant item(s) previously identified that have been corrected; II. Non-compliant item(s) previously identified for which time remains for correction; III. Non-compliant item(s) identified this inspection; IV. Non-compliant item(s) previously identified that have not been corrected:

Assistant Director Cindy Kloster accompanied me on this inspection.

All items in compliance.

8. PREPARED BY *(Signature)*	9. TITLE	10. DATE
Lynn P. Bourgeois	Veterinary Medical Officer	5-13-97
11. COPY RECEIVED BY *(Signature)*	12. TITLE	13. DATE
Robert W. Stout	Clinical Veterinarian	5-13-97
14. REVIEWED BY *(Signature)*	15. TITLE	16. DATE

PART 2 - FACILITY

Animal Usage and Publications for Pennington Center Army Project
October 1996 - October 1997.

Total number of animals used:

640 rats
136 mice

The classification based on those described by our IACUC:
Class C: Drug intervention for pain or distress would interfere with the protocol.

Publications:

Youngblood, B.D., D.H. Ryan, and R.B.S. Harris. Appetitive operant behavior and free feeding in rats exposed to acute stress. Physiol Behav 62: 827-830, 1997.

Harris, R.B.S., J. Zhou, B.D. Youngblood, G.N. Smagin, and D.H. Ryan. Failure to change exploration or saccharin preference in rats exposed to chronic mild stress. Physiol Behav (In Press), 1997.

Rybkin, I.I., Y. Zhou, J. Volaufova, G.N. Smagin, D.H. Ryan, and R.B.S. Harris. 1997. The efect of restraint stress on food intake and body weight is determined by time of day. Am J Physiol 273: R1612-1622,1997.

Zhou, Y., P.D. Elkins, L.A. Howell, D.H. Ryan, R.B.S. harris. Apolipoprotein-E deficiency results in an altered stress responsiveness in addition to an impaired spatial memory in young mice. Brain Research (submitted), 1997.

Absracts:

Rybkin,I.I., Y. Zhou, G.N. Smagin, D.H. Ryan, and R.B.S. Harris. Time dependent effects of restraint stress on food intake in rats. FASEB J 11:A1005, 1997.

Smagin, G.N., R.B.S. Harris, and D.H. Ryan. Antisense oligonucleotides to CRF_2 receptor mRNA attenuate corticotropin-releasing factor induced anorexia in rats. FASEB J 11:A 3661, 1997.

Zhou, Y., R.B.S. Harris, and D.H. Ryan. Effects of repeated restraint on food intake, body weight, spatial memory and open field activity in mice. FASEB J 11: A3665, 1997.

Youngblood, B.D., D.H. Ryan, and R.B.S. Harris. Acute restraint plus water immersion stress and L-tyrosine effect on operant and free-feeding behavior in rats. Neuroscience 1997.

Zhou, Y., P.D. Elkins, D.H. Ryan, and R.B.S. Harris. Impaired memory and altered responsiveness to stress in apolipoprotein-E deficient mice. Neuroscience 1997.

Smagin, G.N., L.A. Howell, R.B.S. Harris, and D.H. Ryan. Intracerebroventricular infusion of urocortin activates cerebral catecholamine and indolamine metabolism in rats. Neuroscience 1997.

Howell, L., R.B.S. Harris, and D.H. Ryan. The effect of naltrexone on food intake and saccharin preference in stressed rats. Neuroscience 1997.

Bramlett, S.B., S.L. Hendry, J. Zhou, R.B.S. Harris and J.J. Zachwieja. Acute exercise reduces leptin expression in retroperitoneal but not epididymal fat. SEACAM 1998.

APPENDIX
TASK 3:
STRESS, NUTRITION AND MENTAL PERFORMANCE

Table 1.
Diet Composition (g/10 Kg)

	Low Fat	High Fat
Casein	1464	2000
AIN 76 Vitamin Mixture	100	100
AIN 76 Mineral Mixture	350	350
DL-methionine	40	40
Alphacel	400	400
Corn oil	262.5	1313
Coconut oil	187.5	937
Starch	3603	2435
Sucrose	3603	2435
% kcal fat	10	40
% kcal protein	16	16

Table 2.
Body Composition (Mean + SE)

	Control	Pair fed	RS
Carcass (g)	383±8	370.8±6	368±7
Fat (g)	40±4	41±2	40±2
Water (g)	244±3	238±3	233±4*
Ash (g)	11.4±0.9	11.6±0.5	10.4±0.6
Protein (g)	97±7	87±2	90±3
Lean body mass (g)	341±9	324±4✠	323±6✠
Water/Lean body mass (%)	71.8±1.2	73.3±0.4	72.1±0.4

* $P<0.05$ compared with control group
✠ $P<0.01$ compared with control group

Table 3.
Serum Assay and Liver Lipid and Glycogen in Rats Fed High Fat Diet and Treated with Restraint Stress or Pair Fed, Measured One Day after the End of Restraint Stress

	Control	Pair fed	Restraint
Insulin (ng/ml)	$1.40^{A} \pm 0.14$	$0.98^{B} \pm 0.14$	$1.62^{A} \pm 0.10$
Glucose (mg/dl)	117 ± 3	114 ± 4	112 ± 3
Corticosterone (ng/ml)	33 ± 9	39 ± 8	28 ± 5
Leptin (ng/ml)	$6.06^{A} \pm 0.84$	$3.63^{B} \pm 0.41$	$5.62^{AB} \pm 0.9211$
non-esterified fatty acid (mEq/ml)	0.75 ± 0.06	0.65 ± 0.03	0.68 ± 0.06
Liver lipid (g/liver)	289 ± 19	274 ± 14	271 ± 17
Liver glycogen (mg/liver)	712 ± 165	362 ± 115	450 ± 87

Data are mean + SE for groups of 10 rats. Statistical significance was determined by analysis of variance. Differences between observations were determined by post hoc calculation of Duncan Multiple Range Tests at $P<0.05$. Values for a specific parameter that do not share a common superscript are significantly different.

Table 4.
Body Composition and Serum Hormones Measured 4 Days After the End of Repeated Restraint

	Saline Quiet	Saline Stress	Antagonist Quiet	Antagonist Stress
Carcass Weight (g)	326^{A}	311^{B}	322^{AB}	314^{AB}
Water (g)	210^{A}	199^{B}	203^{AB}	200^{B}
Ash (g)	11.5	11.2	12.2	12.0
Fat (g)	33.0	29.4	34.1	31.8
Protein (g)	71.9	70.8	72.1	70.3
Corticosterone (ng/ml)	25.2^{A}	13.5^{A}	101^{B}	208^{C}
Insulin (ng/ml)	1.38^{AC}	1.19^{C}	2.10^{B}	1.80^{AB}
Leptin (ng/ml)	3.11^{AB}	2.06^{B}	4.35^{A}	3.38^{AB}

Data are means for groups of 5 or 6 rats. Values that do not share common superscript are significantly different, determined by two-way analysis of variance and post hoc calculation of Duncan Multiple Range test ($P<0.05$).

Table 5.
Effect of Repeated Stress and ICV Injections of αhCRF on Organ Weights of Rats Exposed to Repeated Restraint

Group	ING pad (g)		Epi fat pad (g)		RP fat pad (g)		Liver (g)		Adrenal (mg)	Thymus (mg)	
αhCRF/quiet	2.30	±	1.91	±	0.99	±	9.69	±	46 ± 3	247	±
	0.19		0.14		0.11		0.54			22	
αhCRF/restraint	2.39	±	1.93	±	1.04	±	9.66	±	49 ± 3	219	±
	0.12		0.11		0.07		0.61			28	
saline/ quiet	1.96	±	1.67	±	0.78	±	9.91	±	50 ± 3	277	±
	0.15		0.14		0.09		0.46			28	
saline/ restraint	1.74	±	1.72	±	0.72	±	8.76	±	49 ± 2	228	±
	0.17		0.18		0.12		0.87			32	
2-Way ANOVA											
αhCRF	0.002		NS		0.01		NS		NS	NS	
Restraint	NS		NS		NS		NS		NS	NS	
Interact.	NS		NS		NS		NS		NS	NS	

Additional assays are in progress to determine body composition, serum glucose, insulin and leptin levels, and concentrations of appetite regulating peptides (NPY, CRF) in the PVN of the hypothalamus.

Table 6.
Carcass Composition (g) of Twice Restrained Wistar Rats

	HF Control	HF Rest
Carcass Weight	536	511*
Protein	142	129*
Water	313	302*
Fat	69	69
Ash	12.6	12.0
Corticosterone (ng/ml)	22	22
Leptin (ng/ml)	17	15

Data are means for groups of 7 rats, an asterisk indicates a significant difference between groups, determined by t-test. Measurement were made 13 days after the end of the second bout of repeated restraint.

Table 7.
Physiological Parameters and Neurotransmitters Measured in Sleep Deprived for 96 hours and Fed Control or Valine Supplemented Diet

	cc cld	cc valine	tc cld	tc valine	sd cld	sd valine
24hr food intake (g)	129	105.7	115.2	104.7	122.2	95.5
48hr food intake (g)	126.8	105.6	104.8	92	122	97.1
72hr food intake (g)	116.5	101.2	103.6	84.7	110.1	97.2
96hr food intake (g)	120.1	110.2	104.2	94.1	109.1	106
Insulin (ng/ml)	1.5	1.07	1.07	0.62	0.73	1.06
Thymus wt. (g)	515.7	440.2	370.4	339.2	348.7	343.8
96hr rectal temp.	37.71	37.75			38.75	38.62
Neurotransmitters (ng/mg tissue)	**cc cld**	**cc valine**	**tc cld**	**tc valine**	**sd cld**	**sd valine**
Hypothalamus						
Tryptophan	2.8	2.4	2.95	3.48	3.94	3.48
5-HT	0.92	0.81	0.86	0.91	0.84	0.9
5-HIAA	0.731	0.661	0.972	1	1.07	1.05
5-HIAA/5-HT	0.806	0.82	1.13	1.1	1.27	1.23
Hippocampus						
Tryptophan	2	1.6	2.51	2.71	2.87	
5-HT	0.21	0.21	0.24	0.21	0.24	
5-HIAA	0.35	0.31	0.5	0.59	0.65	
5-HIAA/5-HT	1.81	1.51	2.13	2.96	2.67	

Data are means for groups of 8 rats. Statistically significant differences were determined by two-way analysis of variance with diet and treatment (sd, control or tank control) as independent variables.

Table 8.
Physiological Parameters in Sleep Deprived Rats Fed a Diet Supplemented with Histidine

	CC Control	CC Histidine	TC Control	TC Histidine	SD Control	SD Histidine
Final wt (g)	330^{A}	323^{B}	314^{C}	309^{C}	320^{C}	306^{C}
96 hr Intake (g)	105^{A}	90^{B}	107^{A}	105^{A}	106^{A}	96^{B}
Cort (ng/ml)	17^{A}	21^{A}	169^{B}	140^{AB}	170^{B}	175^{B}
Leptin (n/ml)	7.8^{A}	3.8^{B}	3.2^{BC}	1.5^{C}	$3.3B^{C}$	2.2^{BC}
Thymus Wt (mg)	477^{A}	448^{A}	310^{B}	324^{B}	332^{B}	317^{B}
Rectal temp (°C)	38.3^{A}	37.9^{A}			39.4^{B}	39.3^{B}

Data are means for 8 rats. Values that do not share a common superscript are significantly different at $P<0.05$

Table 9.
Brain Monoamines and Histamine
in Rats Fed Control or Hisitidine Supplemented Diet

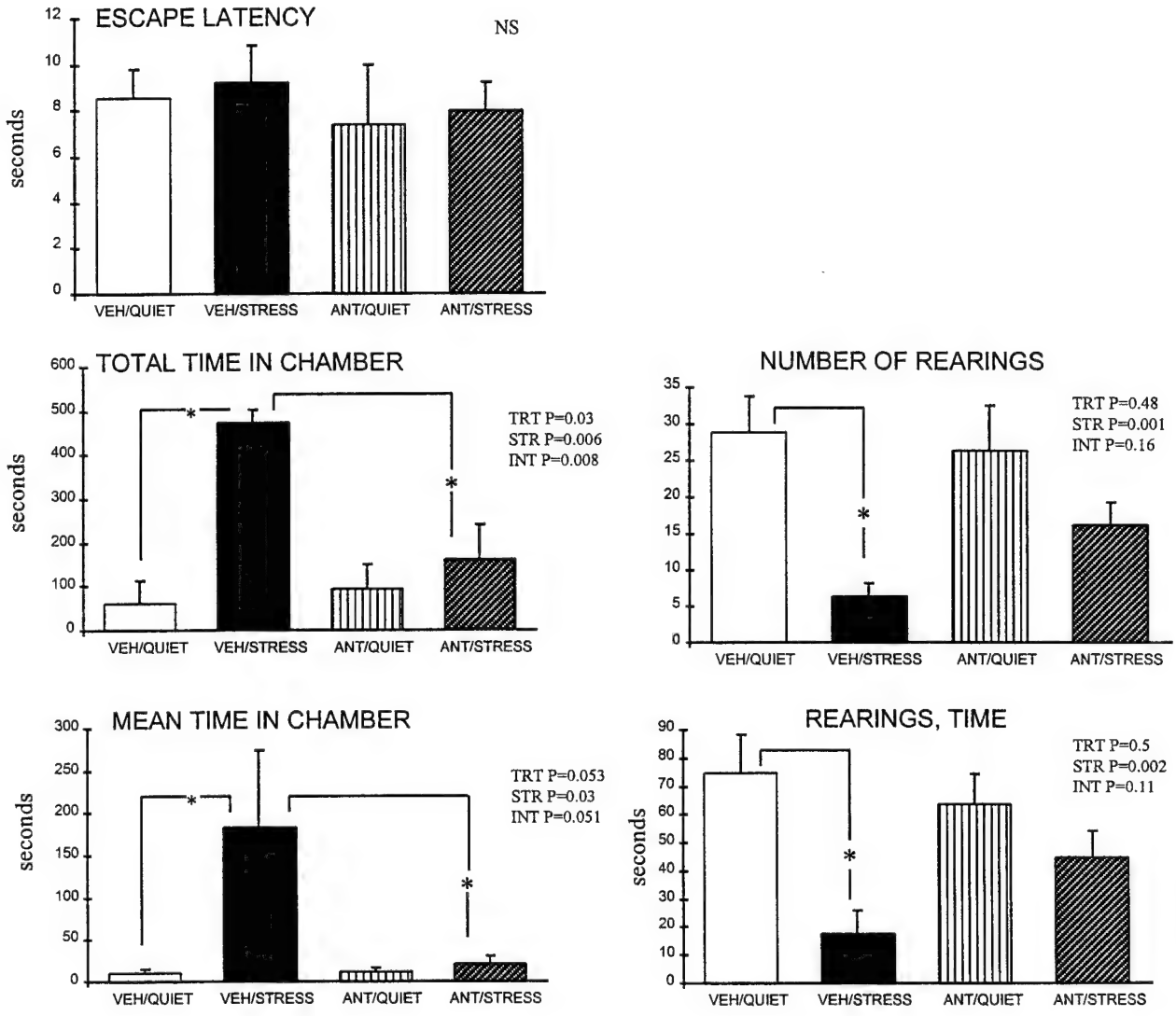
	BRAIN STEM			HIPPOCAMPUS			HYPOTHALAMUS			
	5-HT	**5HIAA / 5HT**	**HISTAMINE**	**5-HT**	**5HIAA /5HT**	**HISTAMINE**	**5-HT**	**5HIAA / 5HT**	**DOPAC/DA**	**HISTAMINE**
C CC	0.30	1.09	2.12	0.04	1.49	1.76	0.76	0.78	0.29	10.41
	0.02	0.14	0.15	0.00	0.18	0.23	0.05	0.08	0.04	0.52
C SD	0.33	1.55	2.87	0.03	2.45	2.13	0.71	1.30	0.34	13.52
	0.01	0.04	0.36	0.00	0.24	0.32	0.07	0.15	0.08	3.32
C TC	0.32	1.43	3.16	0.03	2.71	3.14	0.70	1.19	0.37	15.93
	0.01	0.06	0.76	0.00	0.42	0.74	0.05	0.11	0.10	2.57
H CC	0.32	1.14	7.69	0.04	1.79	9.32	0.81	0.84	0.28	42.82
	0.02	0.06	1.03	0.00	0.13	2.08	0.09	0.06	0.03	3.73
H SD	0.32	1.50	8.13	0.03	2.80	9.89	0.68	1.17	0.34	45.40
	0.01	0.03	1.12	0.01	0.42	1.94	0.08	0.08	0.06	4.77
H TC	0.36	1.42	8.19	0.03	2.81	8.29	0.77	1.10	0.28	43.66
	0.04	0.10	0.68	0.00	0.78	1.19	0.07	0.09	0.03	5.93

Data are means sem for groups of 7 or 8 rats. The brains were collected after 4 days of sleep deprivation.

Table 10.
Fat Pad and Thymus Weights of REMd Rats
(Means ± sem for 8 rats/group)

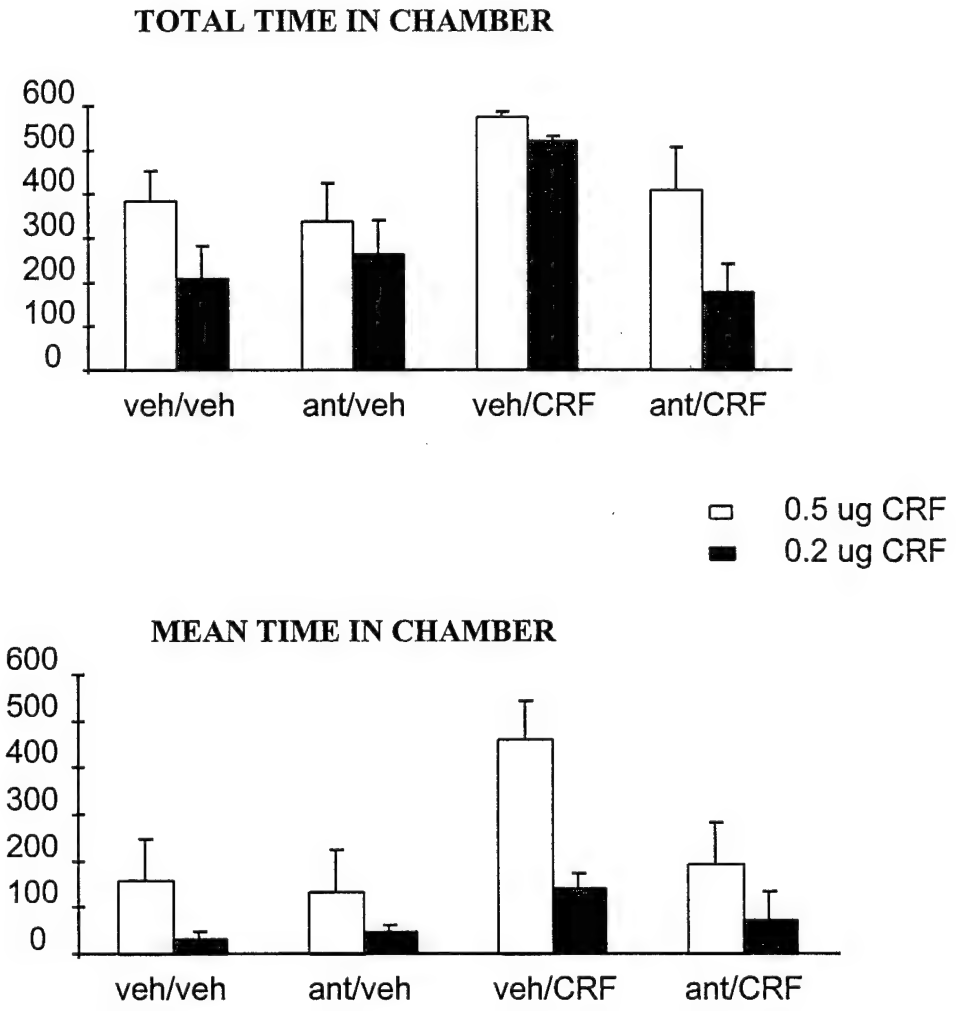
	% Dietary Histidine	Thymus Weight (mg)	Epididymal fat (g)
Cage Control			
	0.24	0.51 ± 0.03	2.4 ± 0.2
	1.125	0.42 ± 0.02	2.3 ± 0.2
	2.25	0.47 ± 0.03	2.3 ± 0.2
	4.5	0.45 ± 0.01	2.1 ± 0.1
REMd Rats			
	0.24	0.41 ± 0.01	2.0 ± 0.2
	1.125	0.37 ± 0.01	2.1 ± 0.1
	2.25	0.39 ± 0.01	2.2 ± 0.2
	4.5	0.44 ± 0.02	2.4 ± 0.1

Figure 1: The effect of stress and CRF_1 receptor antagonist, NBI27914, on defensive withdrawal behavior.



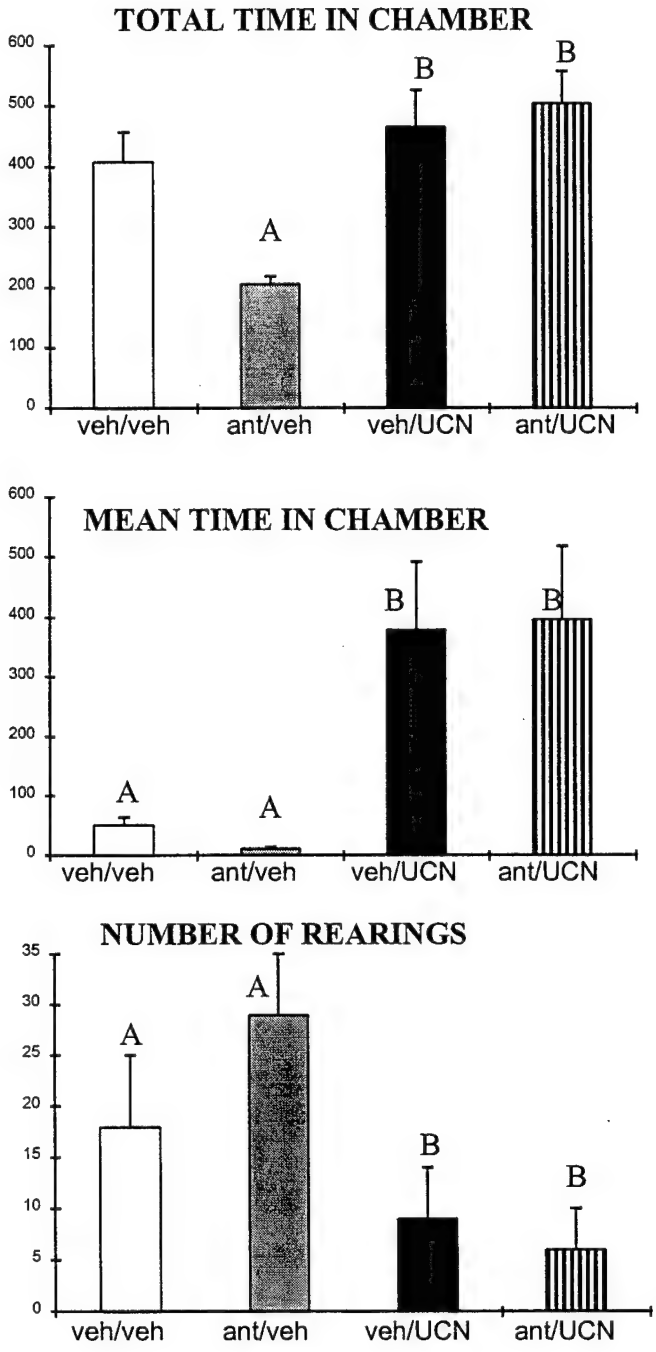
Rats (n=6 per group) familiar with the behavioral apparatus received s.c. injectios of NBI27914 (5 mg/ kg) or vehicle 1 hour before immobilization stress (20 min). Immediately after stress (80 min after the injection of vehicle) animals were tested for defensive withdrawal behavior (10 min testing session). Statistical analyses: Two-way ANOVA with post-hoc LSD test.

Figure 2
The effect of CRF antagonist (NBI27914) and CRF (0.2 and 0.5 ug icv) on defensive withdrawal behavior.



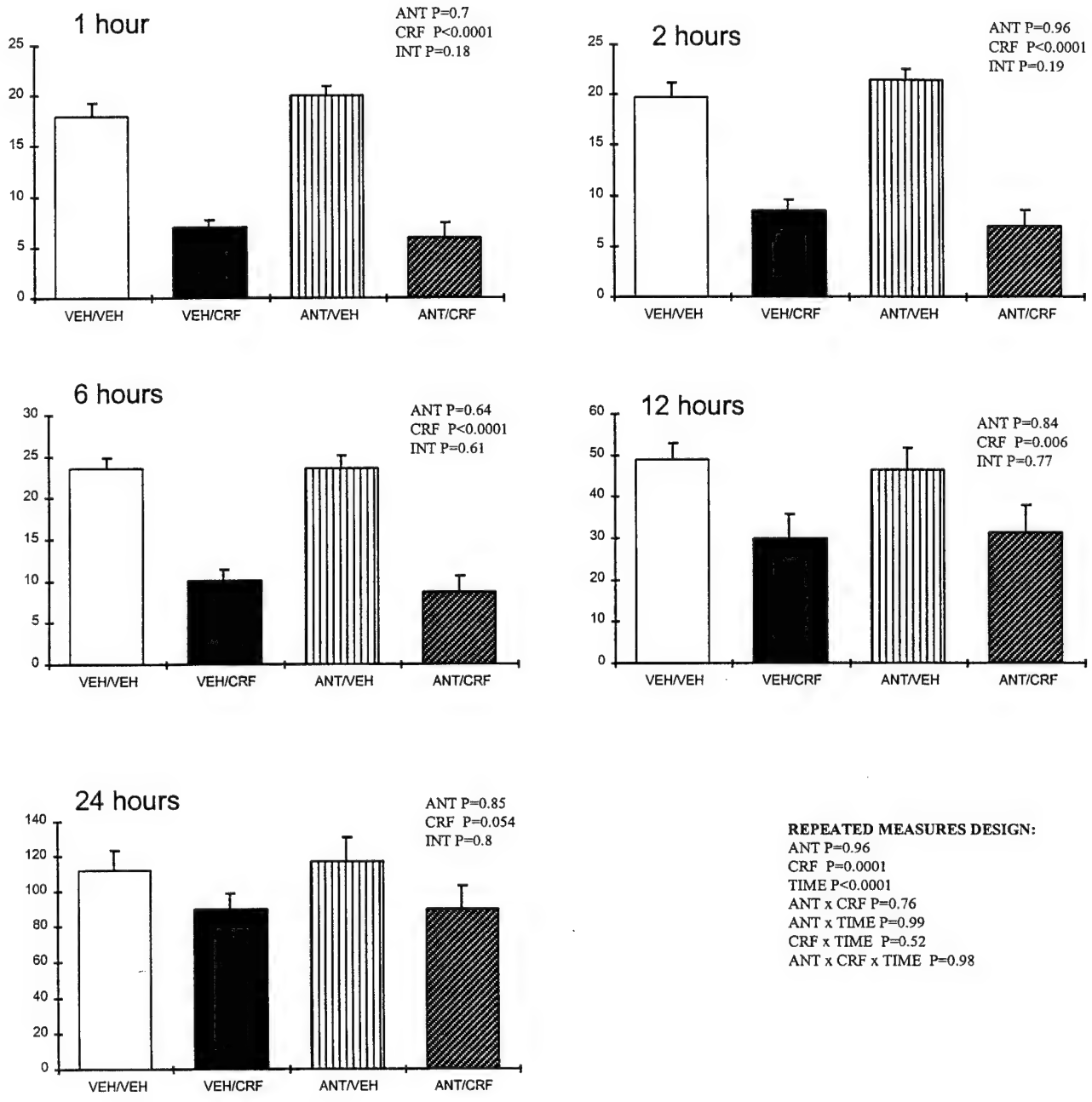
Rats (received s.c. injectios of NBI27c914 (5 mg/ kg) or vehicle 1 hour before icv injection of CRF (0.2 and 0.5 ug).

Figure 3
The effect of CRF antagonis (NBI27914) and UCN (0.2 ug icv) on defensive withdrawal behavior.



Rats (received s.c. injectios of NBI27c914 (5 mg/ kg) or vehicle 1 hour before icv injection of UCN (0.2 ug).

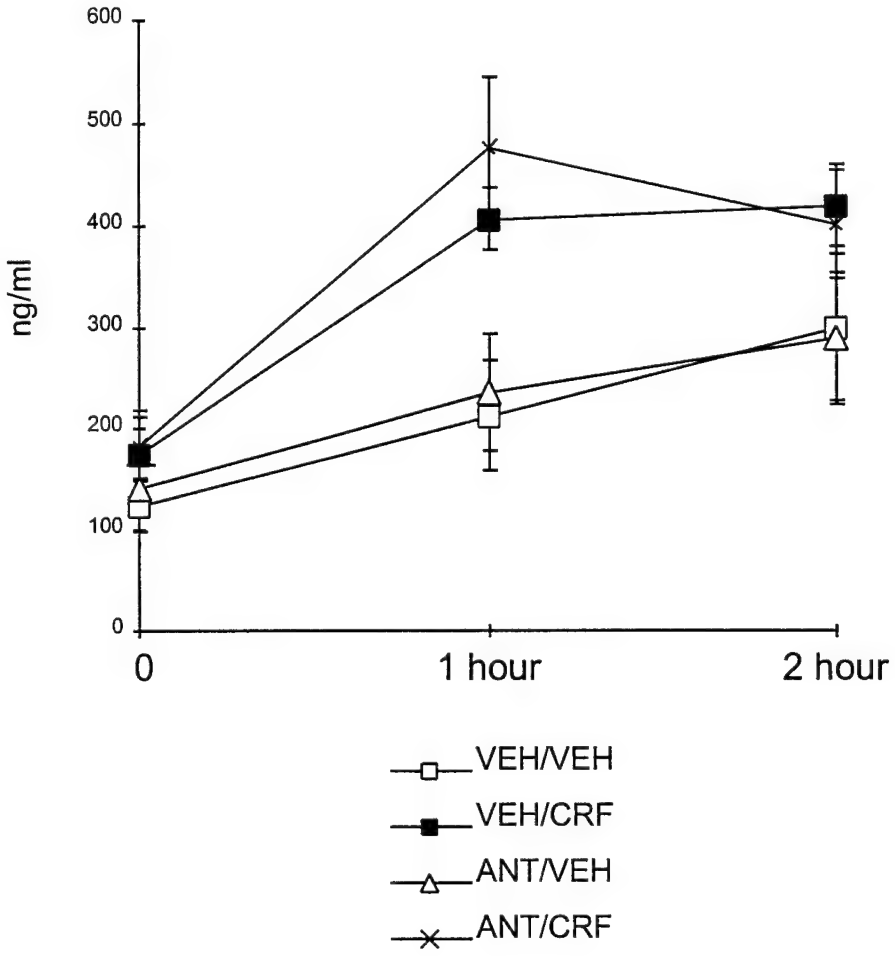
Figure 4. The effect of CRF_1 receptor antagonist on CRF-induced anorexia.



REPEATED MEASURES DESIGN:
ANT P=0.96
CRF P=0.0001
TIME P<0.0001
ANT x CRF P=0.76
ANT x TIME P=0.99
CRF x TIME P=0.52
ANT x CRF x TIME P=0.98

Rats were fasted overnight, injected with CRF_1 receptor antagonist (5 mg/kg s.c.) 1 hour before icv CRF/vehicle injection (3 ug/3 ul).

Figure 5. The effect of CRF 1 antagonist on serum corticosterone in rats infused icv with CRF.



Rats were fasted overnight, injected with CRF_1 receptor antagonist (5 mg/kg s.c.) 1 hour before icv CRF/vehicle injection (3 ug/3 ul).

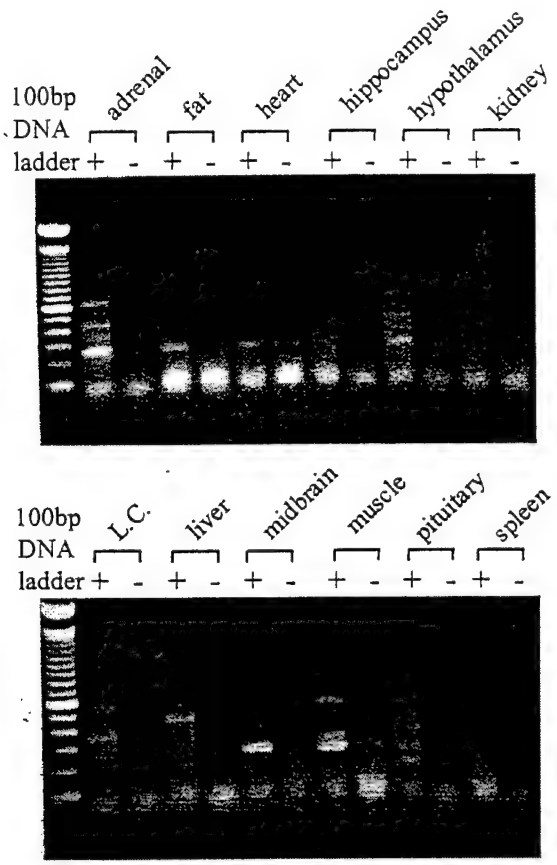
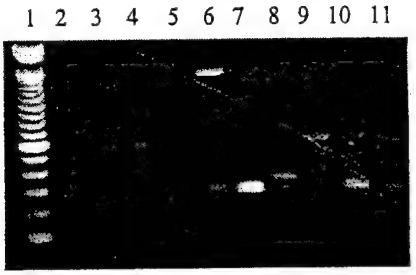
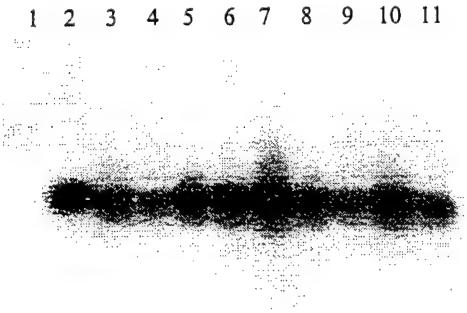


Fig. 6; RT-PCR in various rat tissues showing expression of urocortin gene in both brain and peripheral tissues. Tissues that showed PCR products of the size expected for urocortin were adrenal, fat, heart, hippocampus, hypothalamus, midbrain and pituitary. Expression of urocortin was not detected in liver, spleen, locus coeruleus (LC) or kidney. The "+" and " -" represent the presence or absence of reverse transcriptase in the RT reactions.



a)



b)

Fig. 7: a) RT-PCR of urocortin products (251 bp) in different rat tissues. b) Southern blot of the same PCR products shown in (a) probed with urocortin cDNA. Samples are 100bp ladder (1), urocortin insert (2), adrenal (3), cerebellum (4), fat (5), heart (6), midbrain (7), muscle (8), hippocampus (9), hypothalamus (10), and pituitary (11).

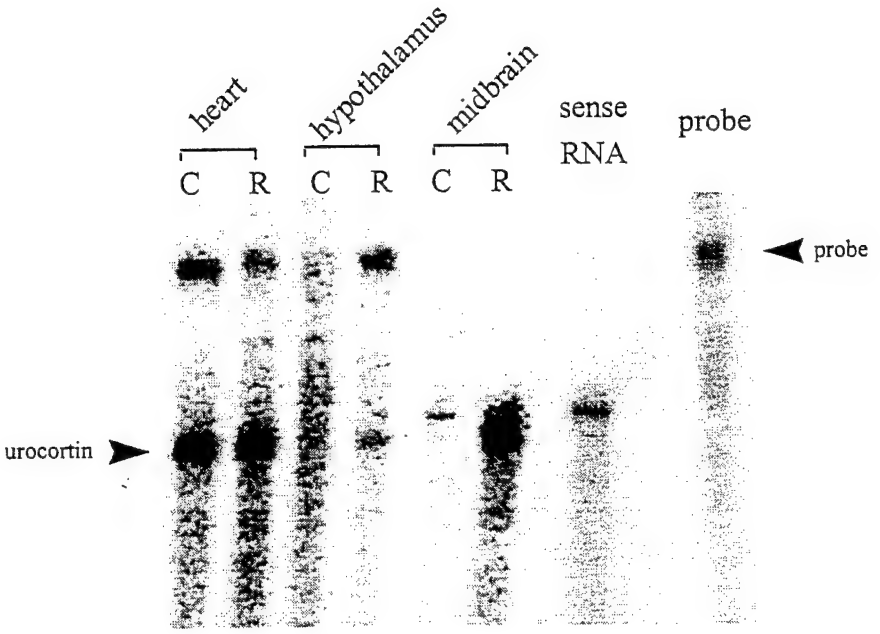
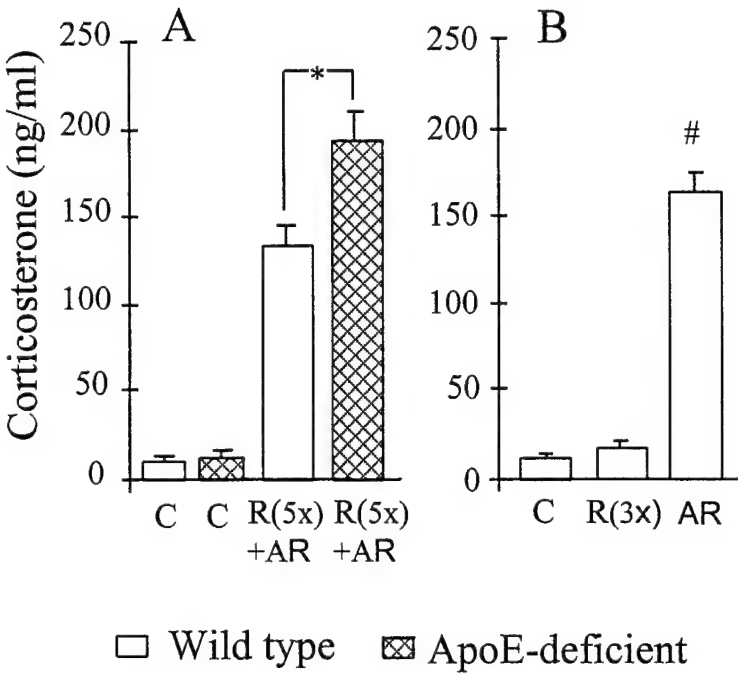


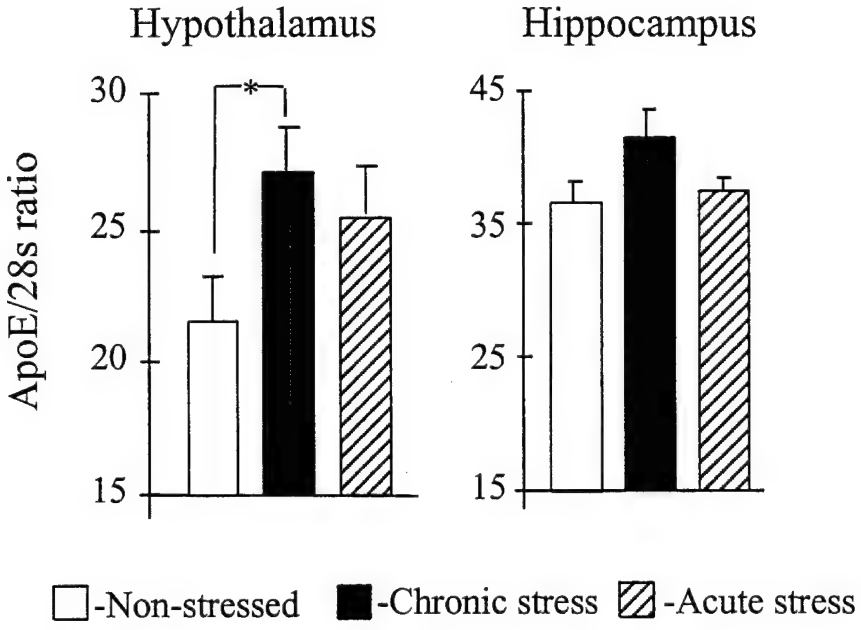
Fig. 8 Detection of urocortin mRNA in tissues from control (C) and restrained (R) rats by RNase protection assay. An increased level of urocortin mRNA was found in heart, hypothalamus and midbrain in rat exposed to 1-hr restraint stress. Note: the protected fragment in sense RNA is about 20 bp longer than the actual protected urocortin fragment in sample RNA due to hybridization within the flanking region of the in vitro transcribed sense RNA with the riboprobe.

Figure 9: Corticosterone response to acute and chronic restraint stress in mice



Data are means + sem for 7 mice. A) ApoE-deficient mice showed a stronger corticosterone response than wild-type mice, to an acute restraint with a same stressor that they exposed 5 times previously (R5x + AR), as indicated by * ($p < 0.05$). B) The corticosterone level was elevated in the wild-type mice exposed to a single acute restraint stress (AR), as indicated by #. There was no difference in corticosterone levels between the chronically stressed mice (R3x) and the non-stressed controls (C).

Figure 10. Effects of restraint stress on ApoE mRNA expression



ApoE mRNA expression in hypothalamus (A) and hippocampus (B) of the wild type mice from Experiment 2, determined by the ApoE/28S ratio (Mean + SEM, n=7). ApoE mRNA expression was up-regulated in hypothalamus ($p < 0.05$, indicated by *) and to a lesser extent, in hippocampus ($p = 0.057$) of the mice killed 24 hr. after the end of a 3-day chronic stress period (20 min restraint per day).

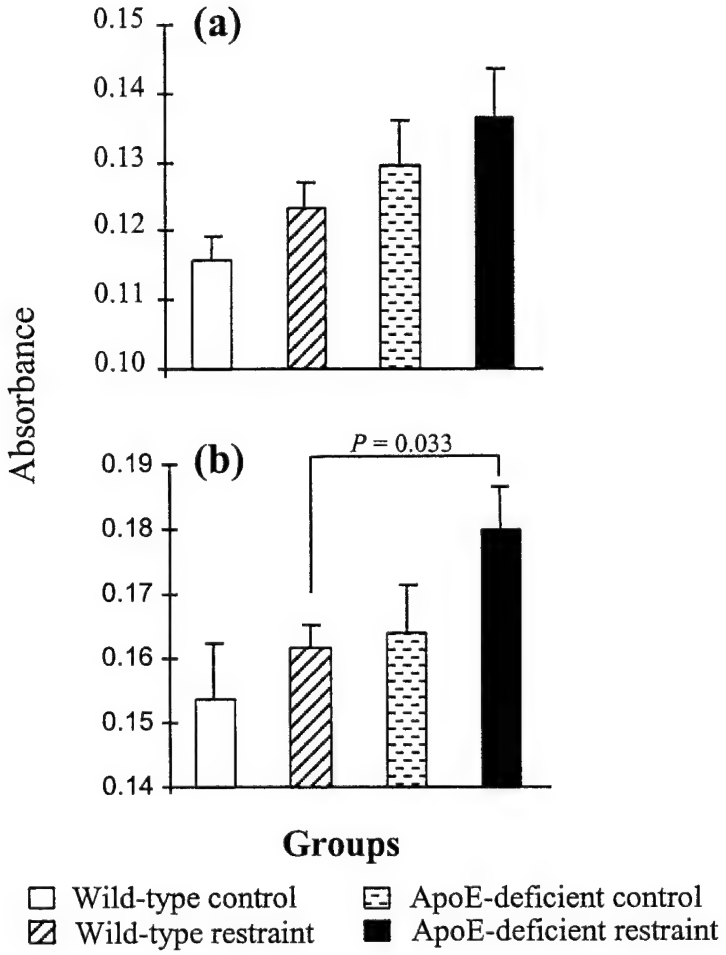


Figure 11. Titers of autoantibodies (IgGs) from sera of both genotypes of mice at age of 7-8 weeks (a) or ~12 weeks (b), as determined by ELISA using the brain homogenates of wild-type mice. Data represent mean + SEM (n=7)

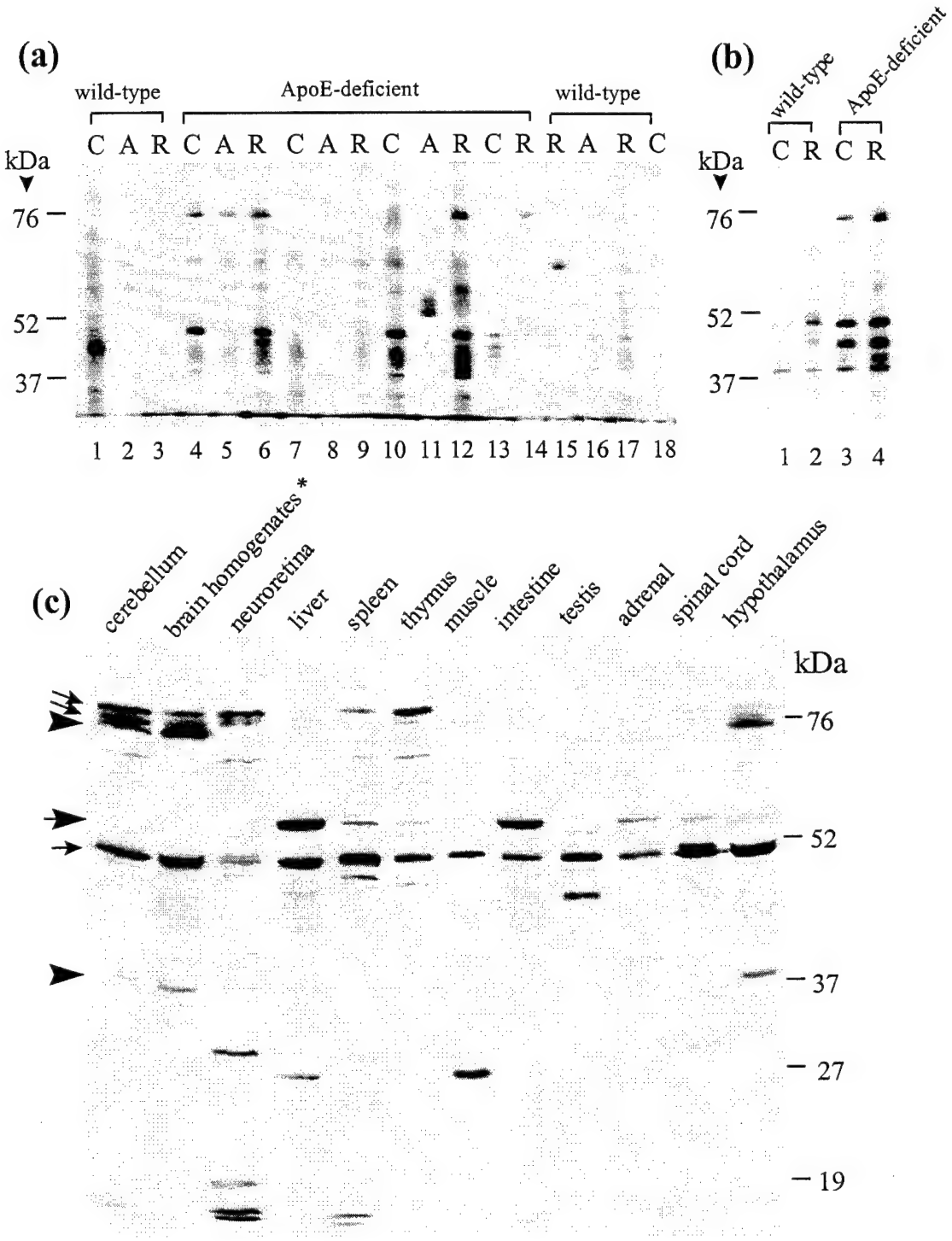


Figure 12 Western blots showing autoantigens recognized by sera from the two genotype of mice at of 7-8 weeks (blot a) or ~12 weeks of age (blot b), and tissue distribution of autoantigens detected by autoimmune sera of ApoE-deficient mice (blot c). Serum from individual mouse, including non-restraint stress controls (C) and those exposed to an acute restraint stress (A) and repeated restraint stress (R), was blotted separately in each lane as numbered at the bottom of the blots (a) and (b). The large arrow indicates a polypeptide (~58 kDa) that was abundant in non-brain tissues. Two brain-abundant antigens (~75 kDa and ~40 kDa) are indicated by arrowheads. * brain homogenates were prepared from whole brain without the cerebellum and brain stem.

Neuronal cells and cellular components recognized by the sera of ApoE-deficient mice (~12 weeks old)

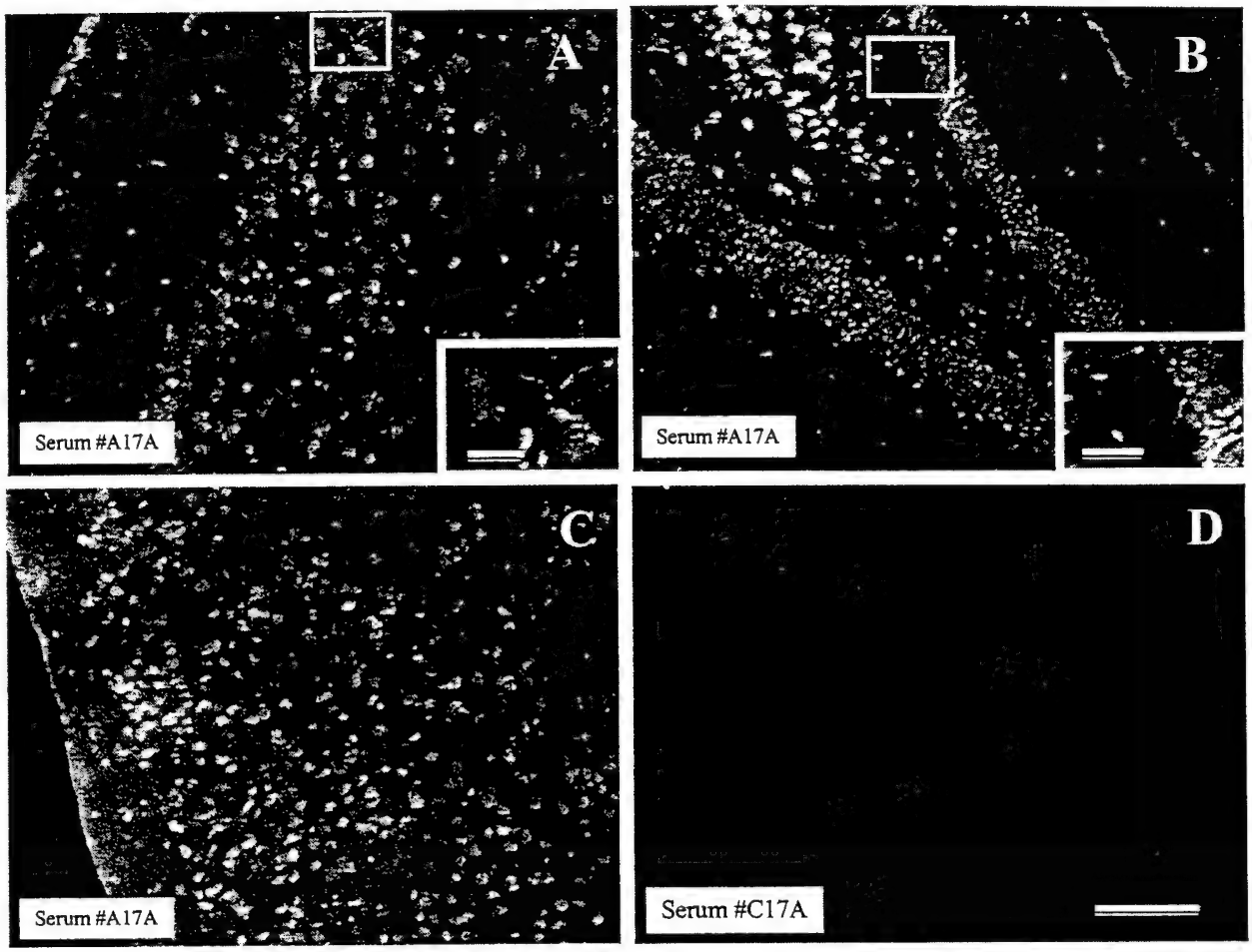
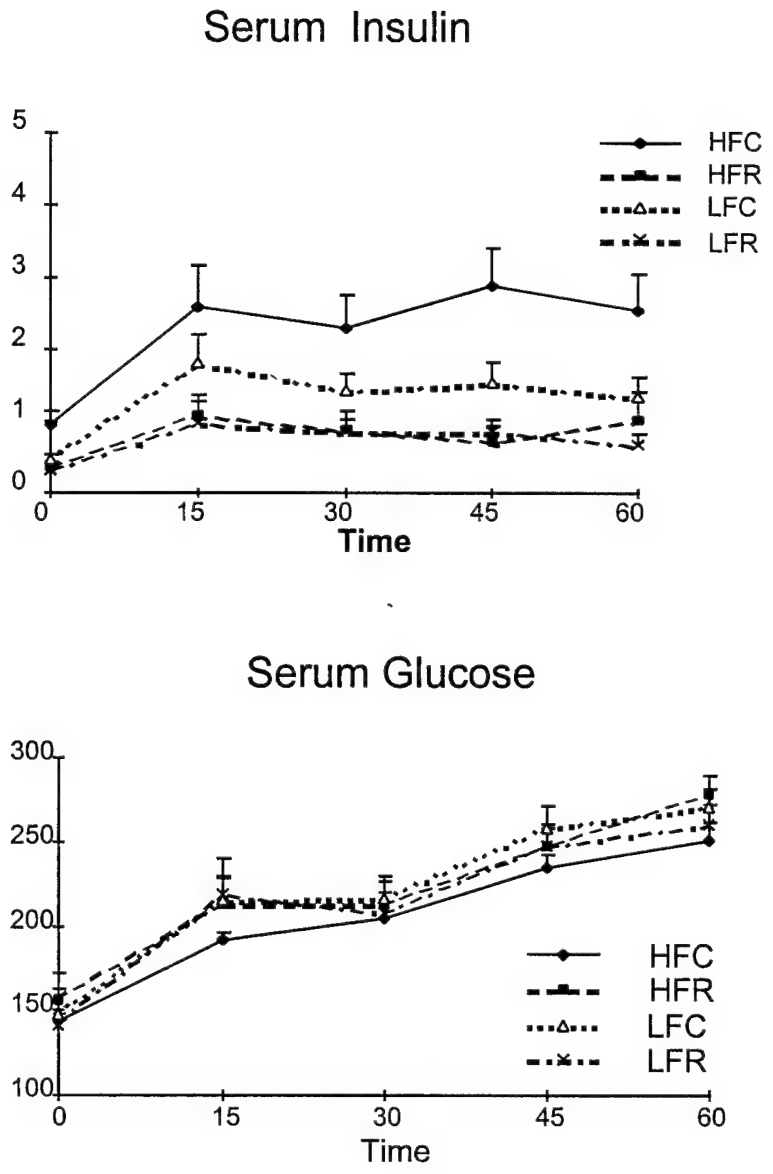


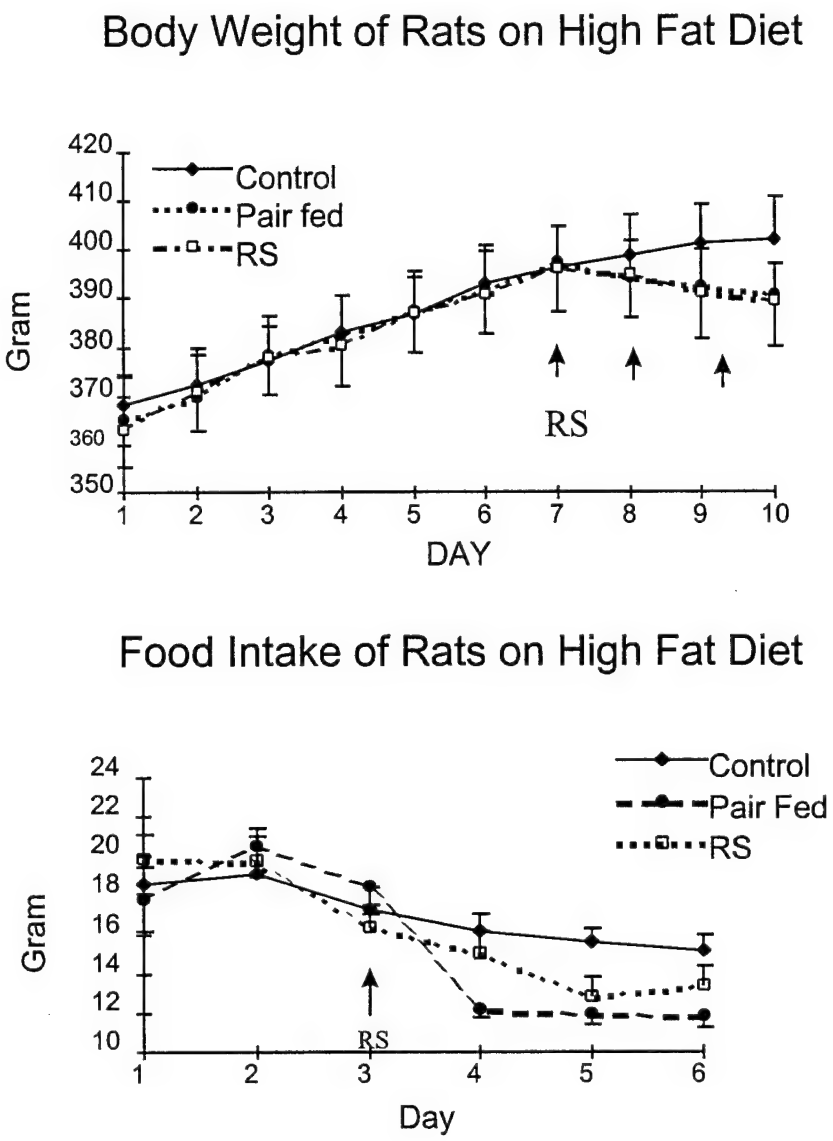
Figure 13. Immunofluorescence confocal micrographs showing the reactivity of sera from ApoE-deficient (A-C) and wild-type (D) mice (~12 weeks old). Strong signals were seen from the nuclear antigens of different types of neuronal cells in different brain areas, including frontal cortex (A), hippocampus (B), and hypothalamus-cortex (C). The autoantibodies also detected the neuronal fibers shown in the boxed regions, which are shown at higher magnification in the inserted micrographs. The serum from a wild-type mouse did not react to the brain cells (D). Bars, ~100 m and ~25 m (inserts)

Figure 14.



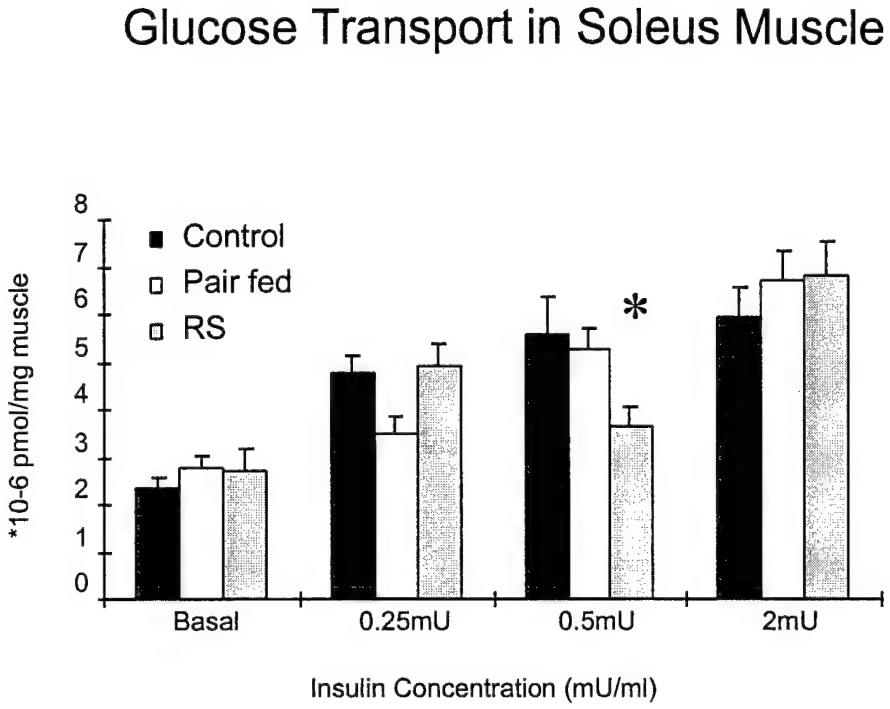
Serum insulin and glucose levels in glucose tolerance test measured one day after 3 hours restraint stress for 3 consecutive days. Dat are means ± sem for groups of 9 rats.

Figure 15.



The body weight and food intake of the rats exposed to repeated restraint. Data are means ± sem for groups of 10 rats.

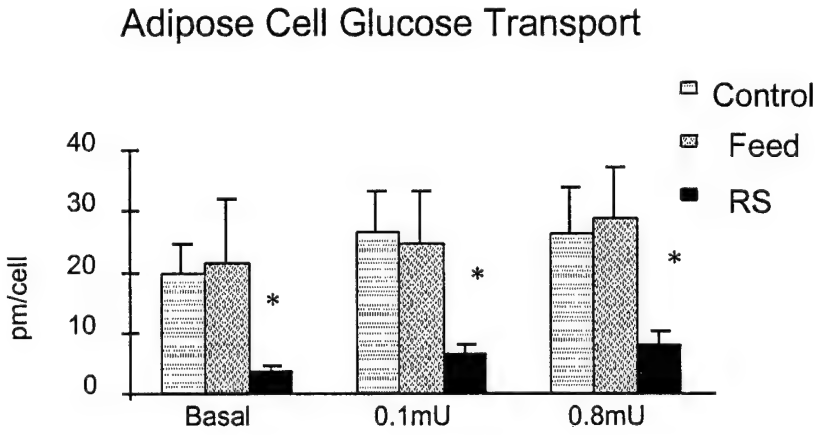
Figure 16.

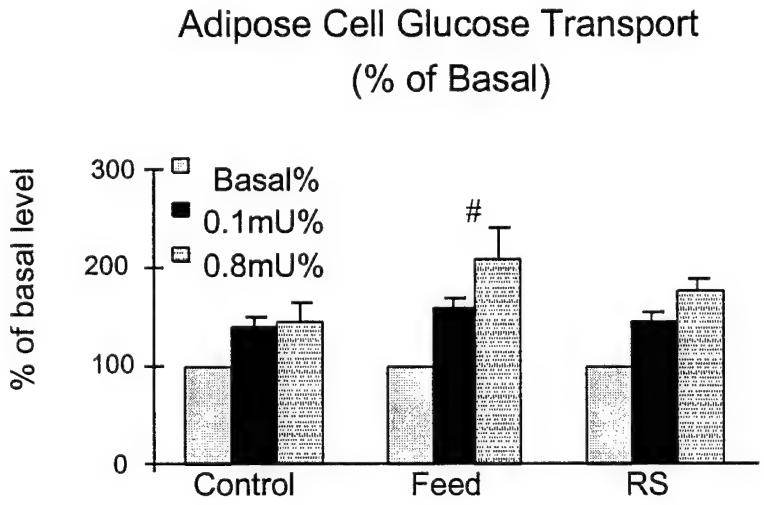


Muscle glucose uptake in rats measured one day after the end of repeated restraint

* P <0.05 Compared with control group

Figure 17.

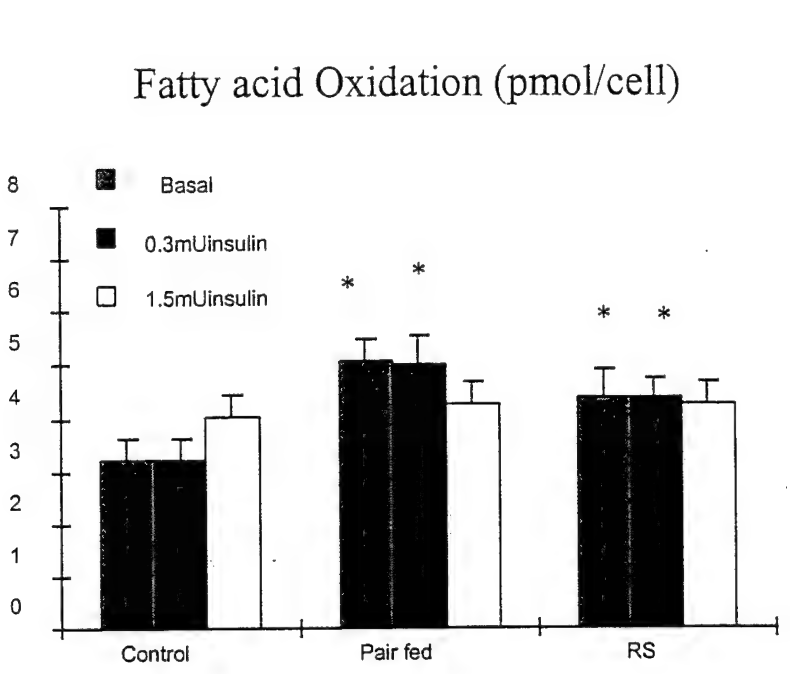




Adpocyte glucose uptake in rats measured one day after the end of repeated restraint

* P<0.05 compared with control and pair feeding groups

# P< 0.05 compared with control and RS groups



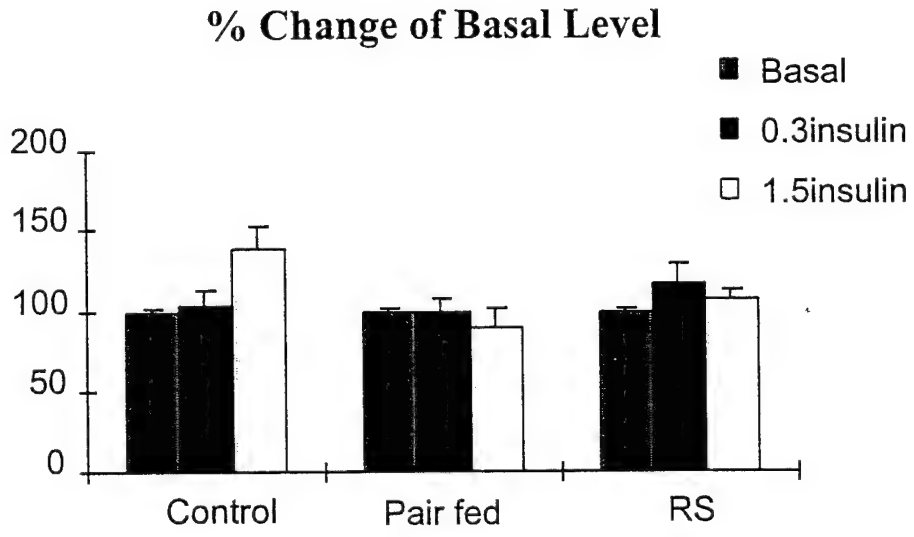


Figure 18 : Fatty acid oxidation in adipocytes of rats fed high fat diet and exposed to 3 hours of restraint stress for 3 consecutive days

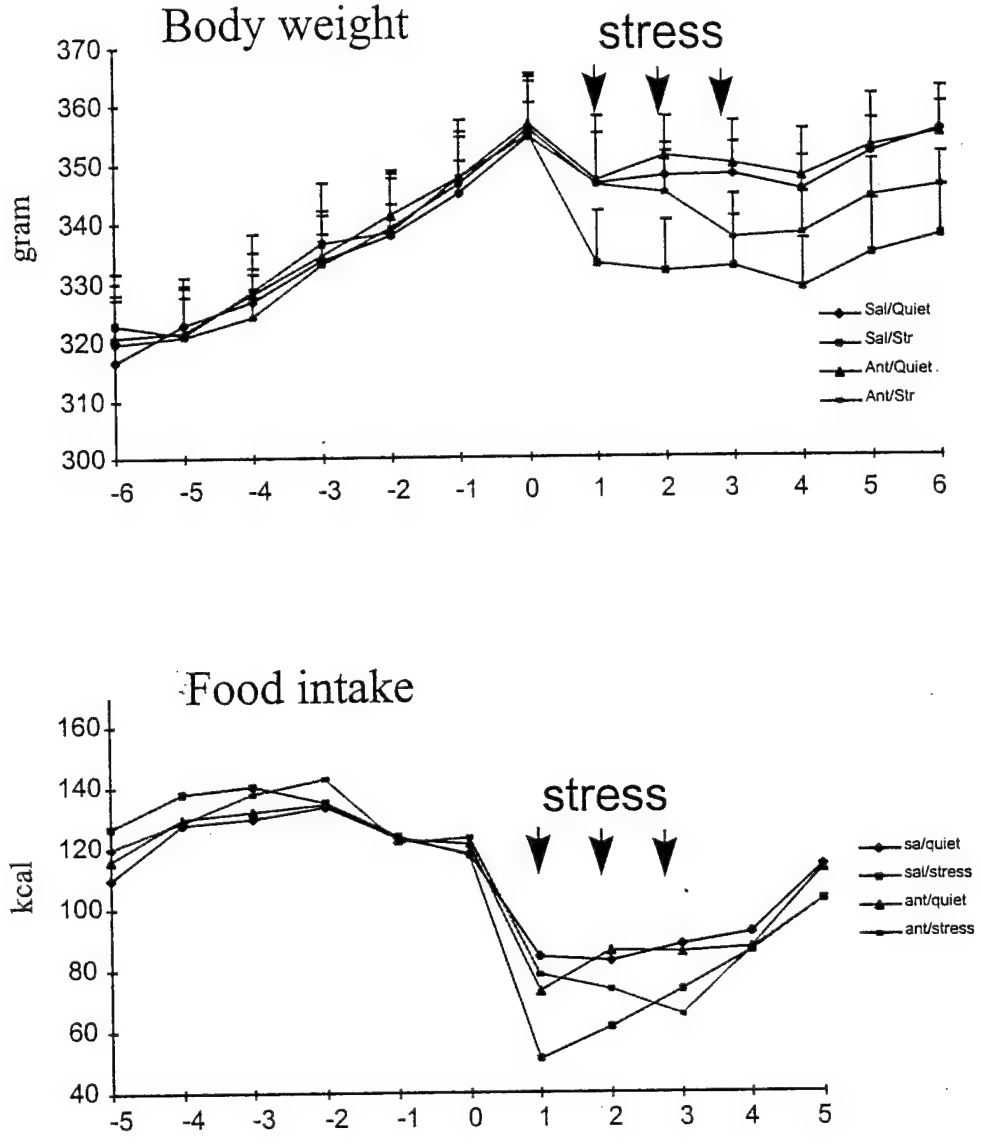


Figure 19. The effect of repeated restraint and administration of CRF receptor antagonist on daily body weight(upper panel) and food intake (lower panel) of rats. Daily restraint sessions (3 hour duration) are indicated by arrows. n=5-6 per group.

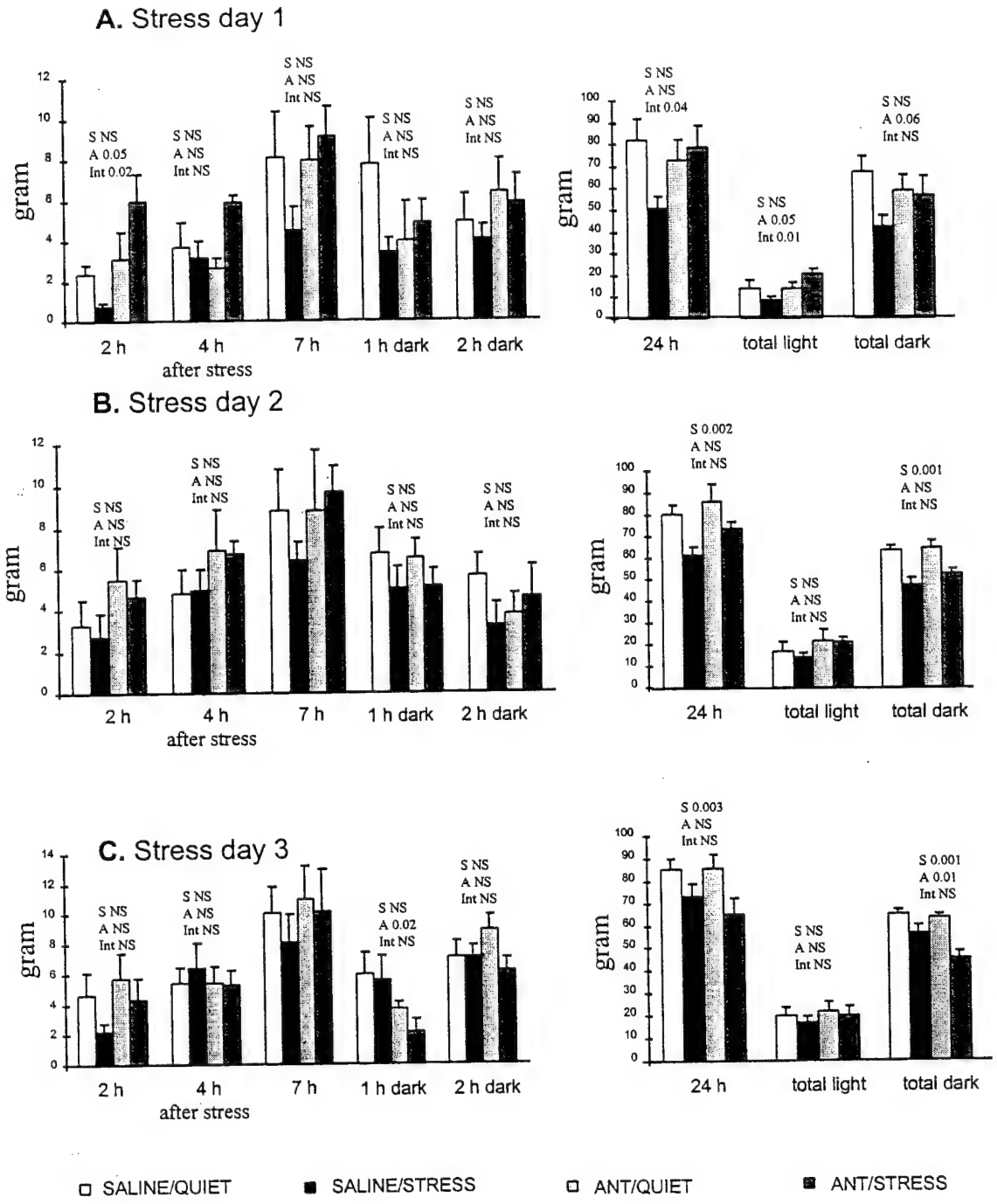


Figure 20. The effect of restraint and CRF antagonist on food intake in rats. Rats were restrained for 3 hours and received access to a liquid diet. Food intake was measured at 2,4and 7 hours after stress, 1 and 2 hours after the onset of dark (8 and 9 hours after stress). The results of a 2 way ANOVA are indicated above the each data point. There were 5-6 animals in each group.

Figure 21
The effect of CRF antagonist (10 ug in the 3rd ventricle)and repeated restraint stress on body weight and food intake.

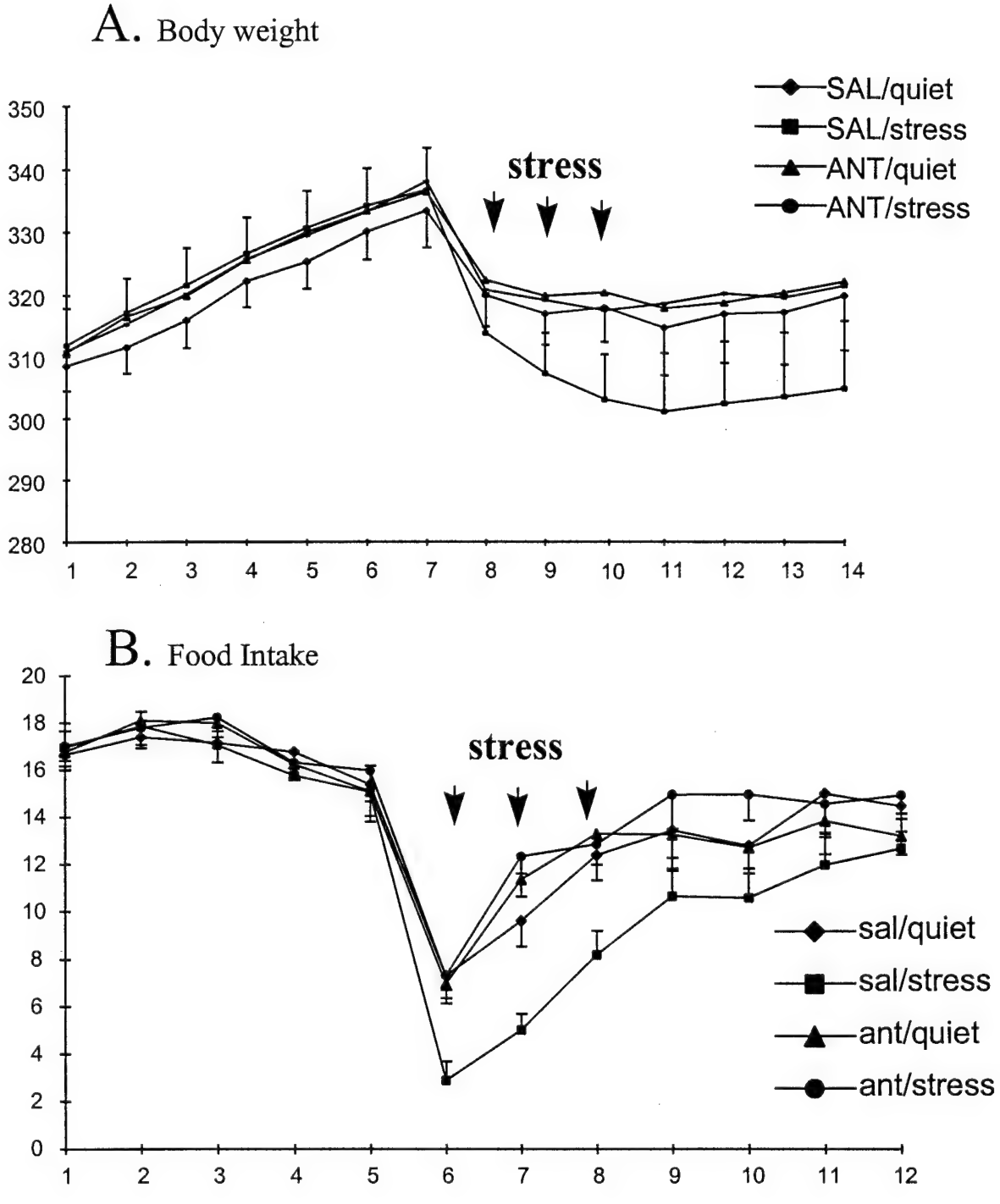


Figure 22
The effect of CRF antagonist (10 ug in the 3rd ventricle)and repeated restraint stress on serum corticosterone.

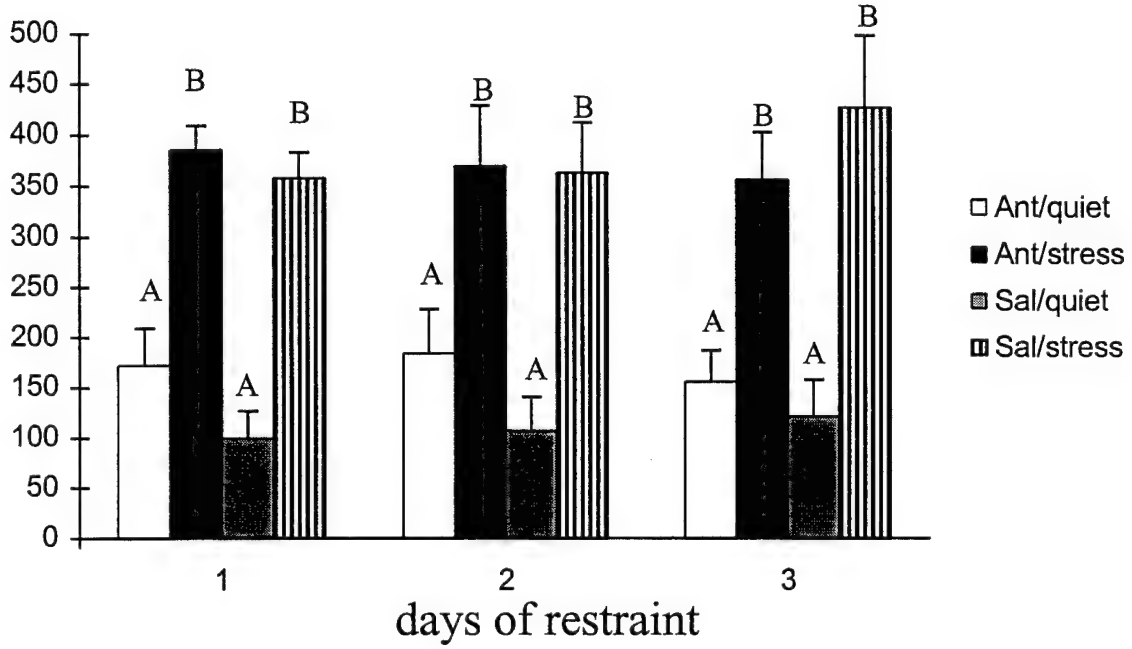
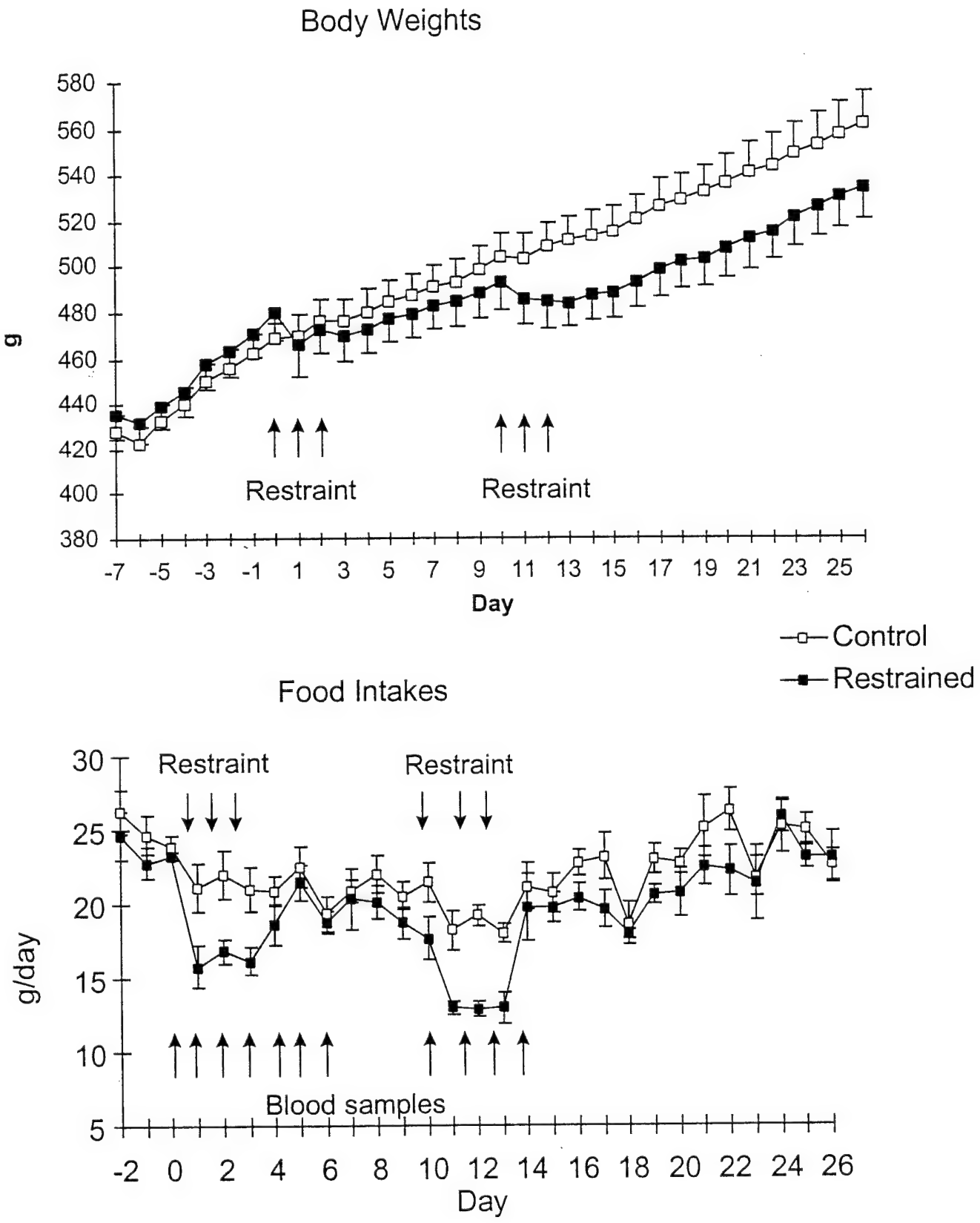
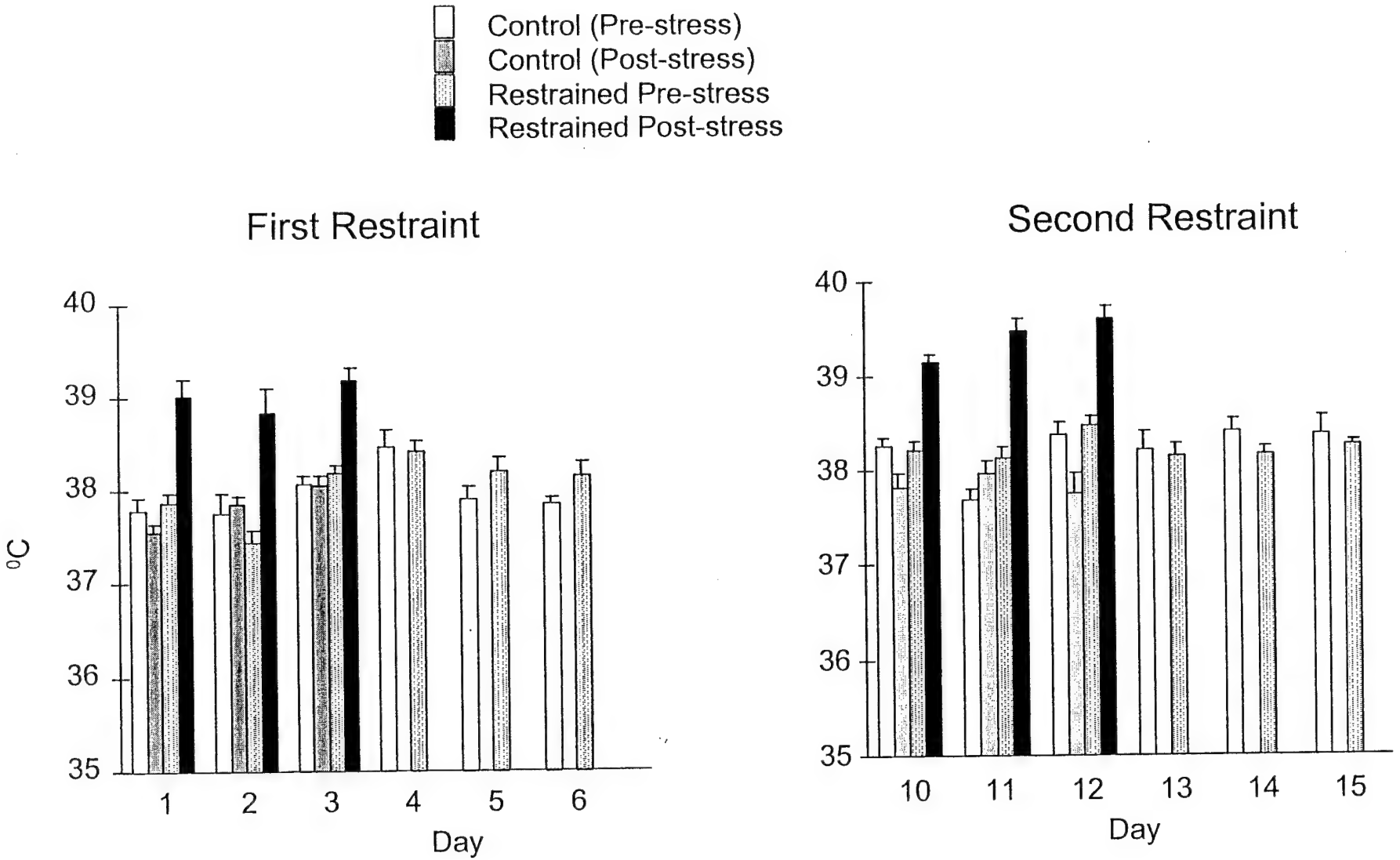


Figure 23: Body Weights and Food Intakes of Wistar Rats Exposed to Two Bouts of Repeated Restraint



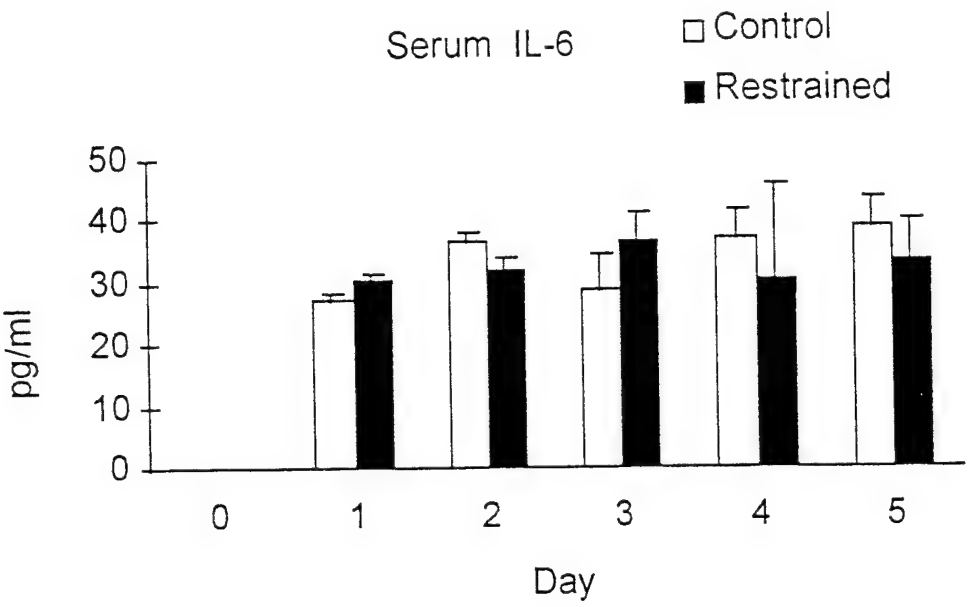
Data are means ± sem for groups of 7 rats

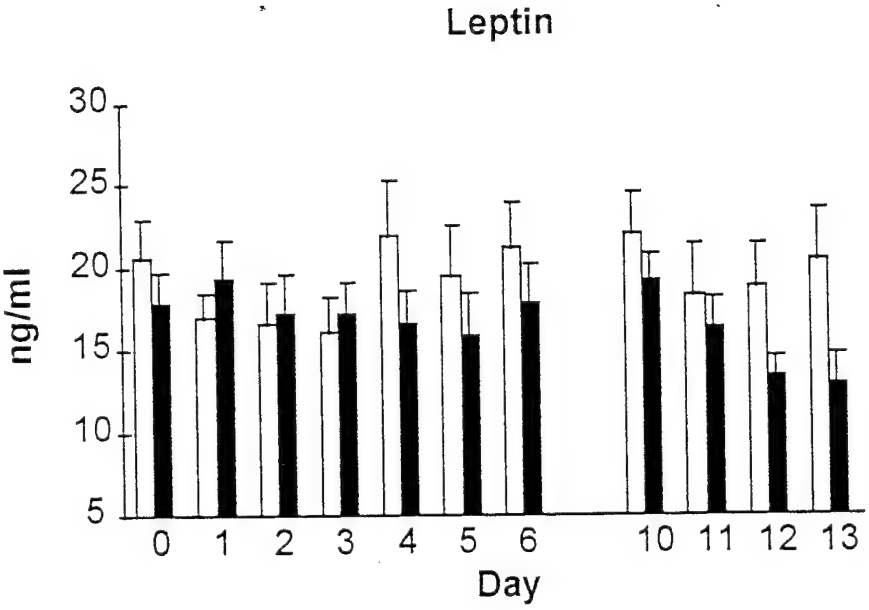
Figure 14: Serum Cytokines in Wistar Rats Exposed to Two Bouts of Repeated Restraint



Data are means + sem for groups of 7 rats. Rectal temperatures were measured before and after stress on each day of restraint and at a time equivalent to the end of stress (10.00 a.m) on other days.

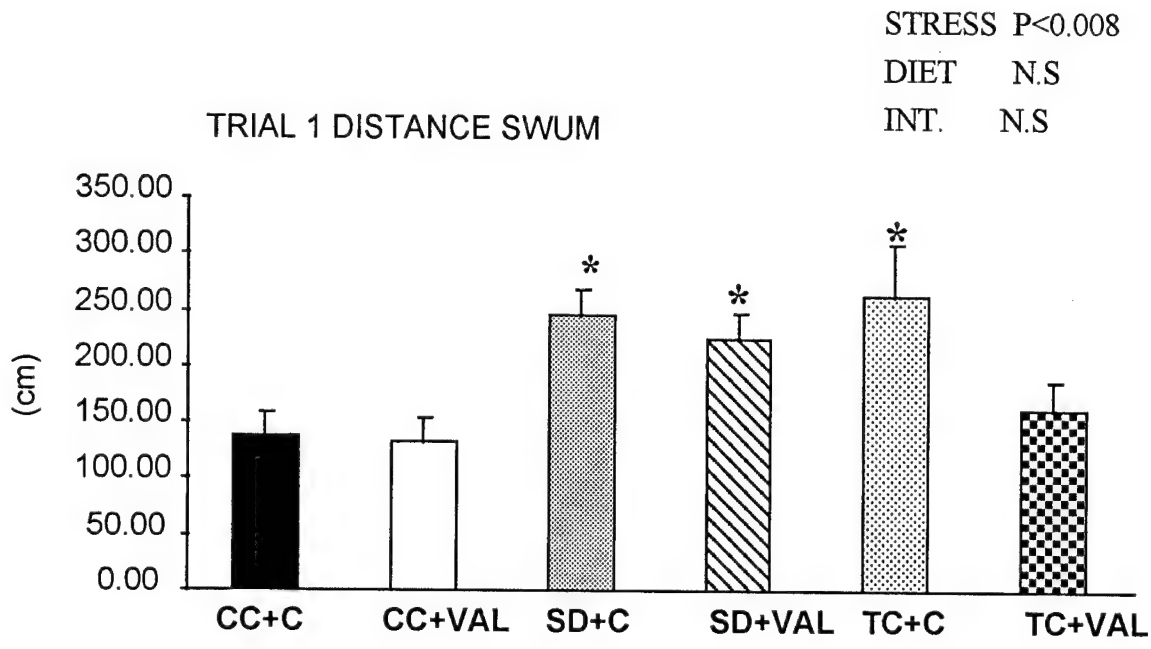
Figure 25: Serum cytokines in Wistar rats exposed to two bouts of repeated restraint

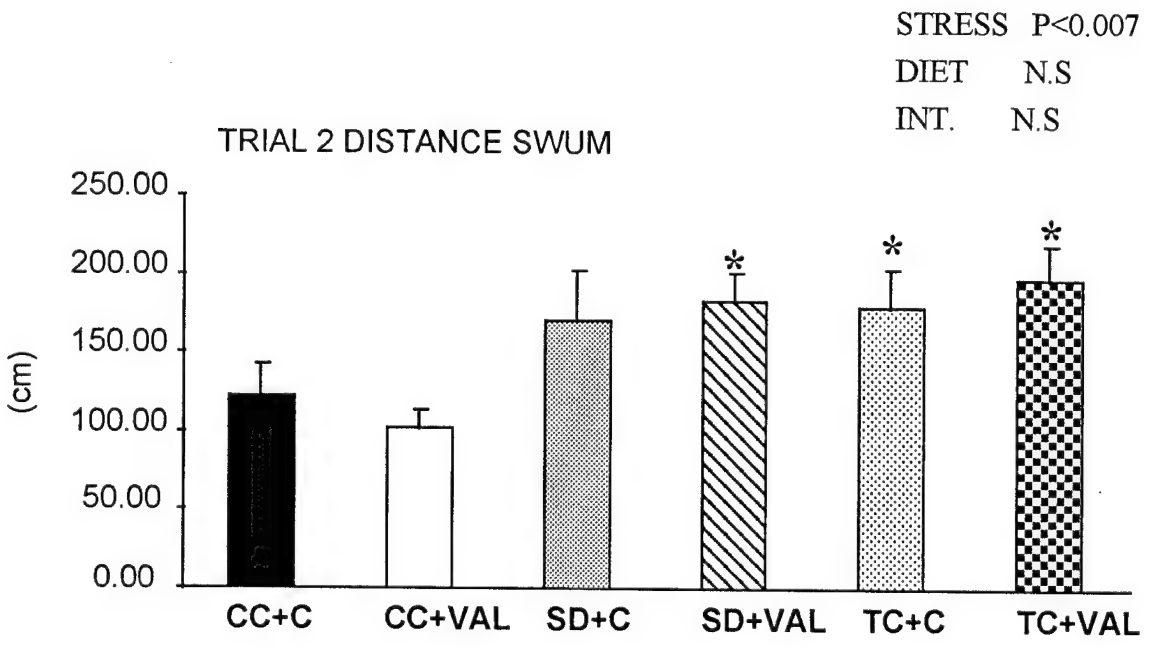




Data are means + sem for 7 rats exposed to repeated restraint on Days 1 - 3 and Days 10 - 12. Blood was collected by tail bleeding one hour after the end of restraint

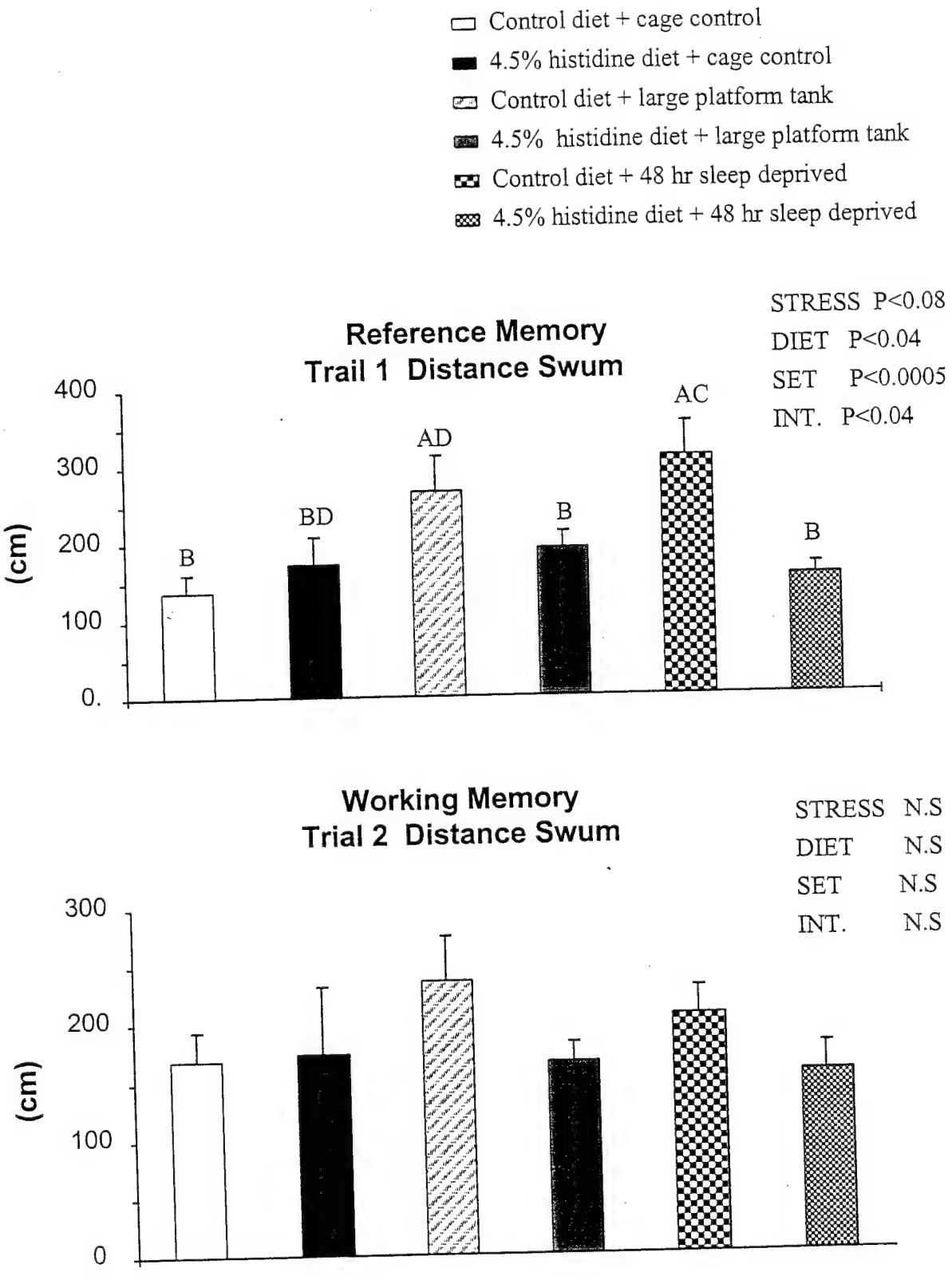
Figure 26: Day 4 of Sleep Deprivation and Stress - Morris Water Maze Place Learning Set Task





Data are means + SEM for groups of 8 rats. * Significantly different from CC + C at P< 0.05, determined by calculation of least-significant differance.

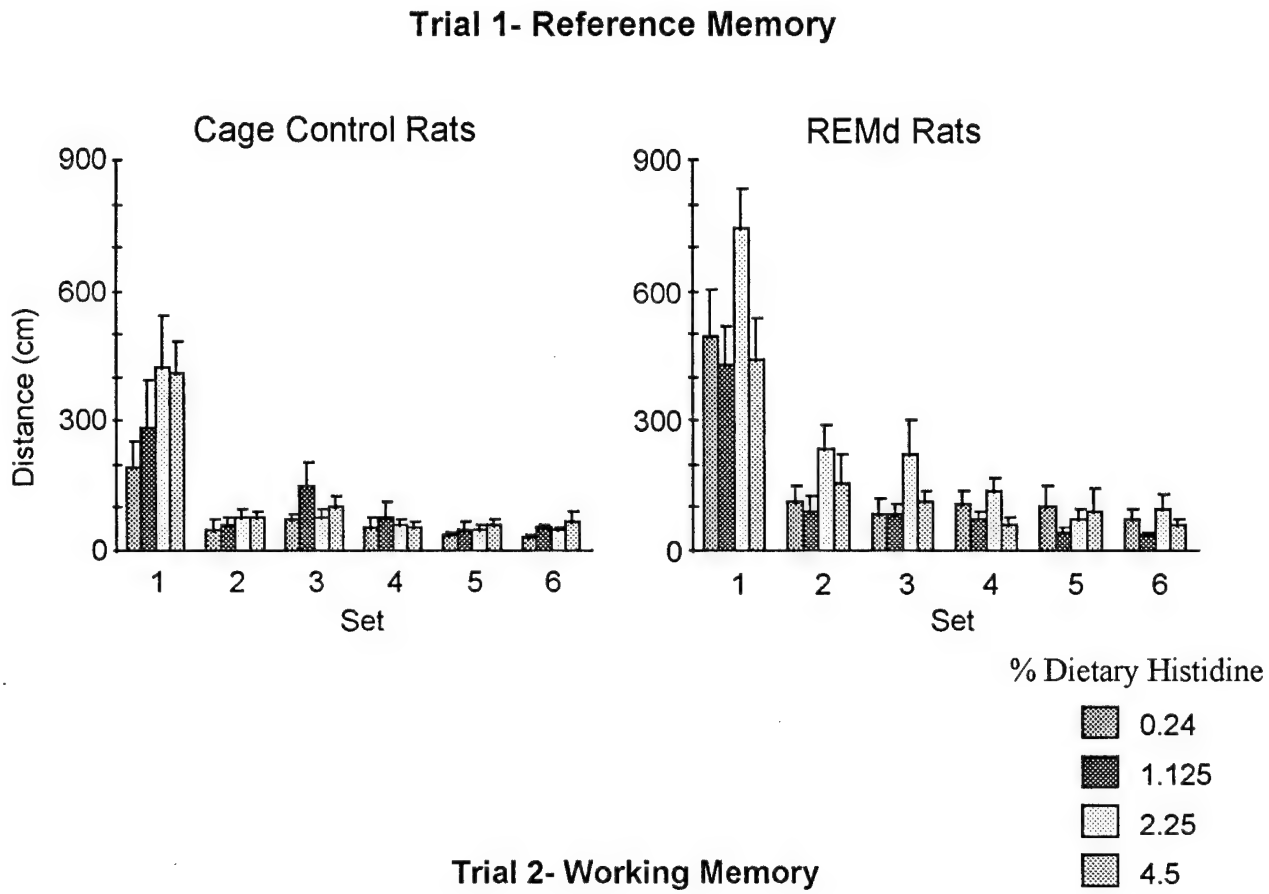
Figure27 Spatial Memory of Rats After 48 Hours Sleep Deprivation

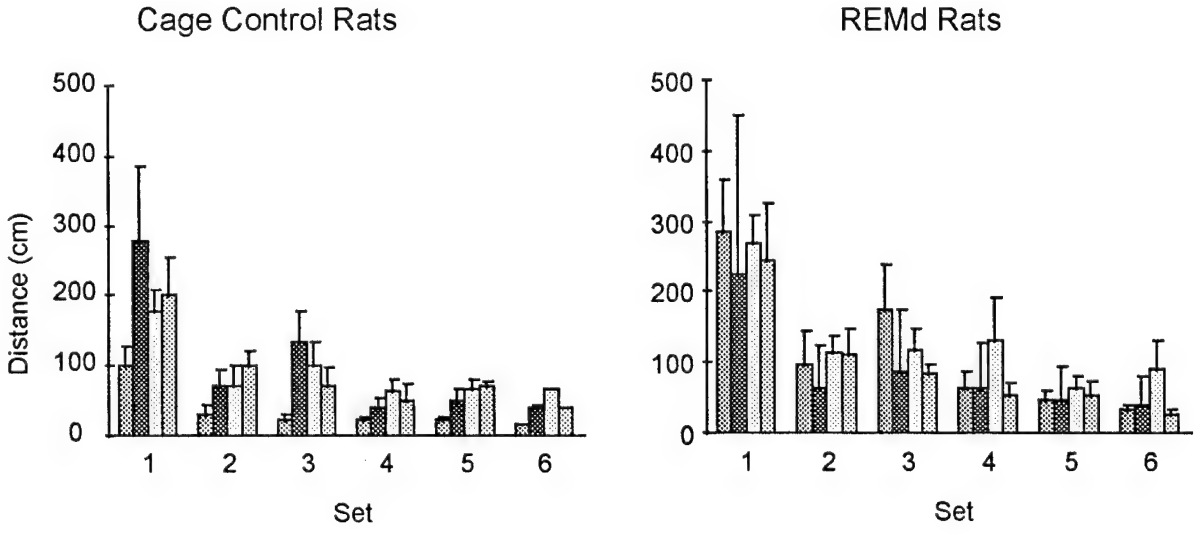


Data are the means + SEM for groups of 8 rats

Values within a specific group that do not share a common letter are significant ($p < 0.05$)

Figure 28: Spatial Memory of REMd Rats Fed Diets Supplemented with Histidine





APPENDIX
TASK 4:
STRESS, NUTRITION AND WORK PERFORMANCE

Table 1.

Men (n=3)	Age (yrs)	Weight (kg)	% Fat (DEXA)	BMI	VO_2 max (l/min)	VO_2 max (ml/kg/min)
1	22	84.6	8.8	27.8	4.298	50.8
2	26	75.4	10.6	25.8	4.694	62.3
3	23	76.2	15.6	24	4.213	55.3
mean ± SE	23.7 ± 1.2	78.7 ± 2.9	11.7 ± 2.0	25.9 ± 1.1	4.402 ± 0.15	56.1 ± 3.3
Women (n=3)						
1	19	55.4	27.4	20.3	3.033	54.7
2	23	64.8	25	21.6	3.440	53.1
3	20	57	28.6	21.5	2.808	49.3
mean ± SE	20.7 ± 1.2	59.1 ± 2.9	27 ± 1.0	21.1 ± 0.4	3.094 ± 0.18	52.4 ± 1.6

Table 2.

Men (n=3)	Baseline (week 1) (kcal/exercise session)	Experimental (week 2) (kcal/exercise session)	Experimental (week 3) (kcal/exercise session)
1	497	499	476
2	404	404	390
3	449	455	469
mean ± SE	450 ± 27	453 ± 27	445 ± 28
Women (n=3)			
1	451	468	465
2	460	458	433
3	427	438	*
mean ± SE	446 ± 10	455 ± 9	449

Table 3.

Men (n=3)	Max Leg Press (lbs)	Max Shoulder Press (lbs)	Peak Anaerobic Power (Watts)
1	400	250	951
2	180	120	655
3	400	190	687
mean ± SE	327 ± 73	187 ± 38	764 ± 94
Women (n=3)			
1	180	50	477
2	210	60	471
3	230	70	430
mean ± SE	207 ± 15	60 ± 6	459 ± 15

Table 4.

Men (n=3)	Muscle Endurance (min)	5 Mile Run Time (min)
1	2:01	45:00
2	2:30	45:59
3	2:10	39:29
mean ± SE	2:13 ± :09	43:29 ± 2:02
Women		
1	3:48	46:45
2	10:27	39:28
3	5:09	48:11
mean ± SE	6:28 ± 2:02	44:48 ± 4:40

APPENDIX
TASK 5:
NUTRIENT DATABASE INTEGRATION LABORATORY

Table 1. Mean Daily Intake of Selected Nutrients During Predeployment, Field Training, and Recovery

	7/16/96		7/17/96		7/18/96		7/19/96		7/20/96		7/21/96		6 Day Ave.		7/31/96		8/1/96		2 Day Ave.	
	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.
N	81		84		86		78		56		53		73	14.6	41		40		40.5	0.7
Energy (kCal)	3328	1466	2959	1451	2780	1393	2892	1828	2839	1839	1841	1300	2773.2	495.9	2735	1505	3196	1521	2965.5	326.0
Protein (g)	126	55	102	56	109	58	98.3	51.8	81.6	42.5	69.6	40.2	97.8	20.0	95.5	48.3	101	49	98.3	3.9
Total Fat (g)	124	60	107	66	97.1	61.0	90.5	55.3	76.0	51.0	75.8	72.5	95.1	18.7	97.1	54.5	98.2	59.2	97.7	0.8
MUFA (g)	47.3	24.0	42.0	25.6	35.4	21.5	34.2	20.1	29.9	21.4	28.6	30.2	36.2	7.2	41.5	24.1	41.3	26.7	41.4	0.1
PUFA (g)	17.7	12.0	16.8	11.4	15.7	13.3	15.0	15.5	13.5	13.2	14.8	17.3	15.6	1.5	11.6	7.7	13.3	7.9	12.5	1.2
Sat. Fatty Acid (g)	45	22	37.3	24.0	34.8	23.3	31.9	19.5	25.2	16.9	24.5	20.7	33.1	7.7	36.5	22.3	35.6	23.4	36.1	0.6
Carbohydrate (g)	411	201	388	197	356	187	348	211	324	203	218	152	340.8	67.5	291	168	385	198	338.0	66.5
Alcohol (g)	13.9	44.9	10.6	43.6	10.4	31.9	45.8	111	80.4	136	4.2	22.1	27.6	29.8	46.9	105.0	56.2	96.6	51.6	6.6
TDF (g)	18.1	10.6	18.3	11.0	17.0	10.1	15.0	10.7	14.2	9.4	11.0	11.4	15.6	2.8	14.0	8.9	16.6	8.6	15.3	1.8
Calcium (mg)	1455	848	1273	1033	1360	1251	1176	825	811	574	700	508	1129.2	305.9	977	743	1118	703	1047.5	99.7
Iron (mg)	20.6	12.8	20.0	16.5	18.2	12.0	17.0	12.6	14.0	8.4	10.9	7.3	16.8	3.7	15.1	8.3	15.3	7.8	15.2	0.1
Magnesium (mg)	385	207	360	212	345	228	326	189	318	208	218	192	325.3	57.8	288	193	339	166	313.5	36.1
Phosphorous (mg)	2011	960	1791	1098	1795	1200	1675	917	1374	727	1072	723	1619.7	339.6	1477	844	1666	764	1571.5	133.6
Potassium (mg)	4528	1920	4314	2399	3737	2141	3365	1718	3062	1568	2242	1420	3541.3	843.6	2559	1417	3183	1347	2871.0	441.2
Sodium (mg)	4370	2160	4096	2419	3695	1811	3482	1710	2998	1993	2831	2314	3578.7	602.1	3066	1574	3271	1794	3168.5	145.0
Zinc (mg)	19.3	11.5	15.1	12.4	15.5	10.1	13.4	8.9	11.5	7.1	9.3	6.0	14.0	3.5	13.7	7.9	12.6	6.4	13.2	0.8
Copper (mg)	2.00	1.09	1.77	1.38	1.69	1.03	1.60	0.98	1.36	0.83	1.10	0.83	1.6	0.3	1.31	0.75	1.44	0.68	1.4	0.1
Vitamin A (IU)	6296	5656	8610	9282	7112	7337	5885	6575	4032	5955	6016	14797	6325.2	1509.2	2715	2891	3385	3328	3050.0	473.8
Vitamin A (RE)	1169	1089	1320	1251	1187	1006	972	941	617	659	828	1510	1015.5	261.4	454	477	554	428	504.0	70.7
Carotenes (mg)	346	410	419	681	458	676	376	587	292	605	411	1438	383.7	59.0	181	222	233	306	207.0	36.8
Vitamin C (mg)	218	231	241	208	200	208	145	180	101	97	77.7	86.9	163.8	66.2	83.5	119.2	119	132	101.3	25.1
Vitamin E (mg)	17.5	47.6	13.1	20.4	11.1	15.0	11.0	12.5	7.0	6.8	6.7	6.0	11.1	4.0	8.5	8.2	7.9	5.3	8.2	0.4
Vitamin B1 (mg)	2.83	3.64	2.62	3.57	2.71	3.40	1.92	1.26	1.69	0.92	1.29	0.90	2.2	0.6	1.68	1.05	1.81	1.02	1.7	0.1
Vitamin B2 (mg)	3.64	3.39	3.29	3.86	3.36	3.88	2.79	1.76	2.29	1.32	1.72	1.14	2.8	0.7	2.09	1.29	2.44	1.08	2.3	0.2
Vitamin B3 (mg)	33.0	20.0	28.5	19.8	28.9	20.8	29.3	18.6	25.4	16.6	18.6	13.4	27.3	4.9	28.3	15.7	29.4	15.5	28.9	0.8
Vitamin B6 (mg)	3.14	3.13	2.63	3.61	2.72	3.59	2.31	1.61	2.28	1.54	1.28	0.78	2.4	0.6	1.91	1.51	2.18	1.24	2.0	0.2
Folacin (mg)	404	246	430	251	398	326	347	253	307	223	192	155	346.3	87.6	268	235	343	223	305.5	53.0
Vitamin B12 (mcg)	8.61	5.58	6.44	6.53	6.66	6.87	5.36	3.98	4.43	3.05	4.15	3.11	5.9	1.7	5.04	3.38	4.98	3.29	5.0	0.0
Cholesterol (mg)	466	265	398	278	398	278	324	224	246	183	263	265	349.2	86.2	285	226	354	301	319.5	48.8

Table 2. Mean Daily Intake of Selected Nutrients for 6 Days

	7/16/96		7/17/96		7/18/96		7/19/96		7/20/96		7/21/96		6 Day Ave.	
	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.
N	81		84		86		78		56		53		73	14.6
Energy (kCal)	3328	1466	2959	1451	2780	1393	2892	1828	2839	1839	1841	1300	2773.2	495.9
Protein (g)	126	55	102	56	109	58	98.3	51.8	81.6	42.5	69.6	40.2	97.8	20.0
Total Fat (g)	124	60	107	66	97.1	61.0	90.5	55.3	76.0	51.0	75.8	72.5	95.1	18.7
MUFA (g)	47.3	24.0	42.0	25.6	35.4	21.5	34.2	20.1	29.9	21.4	28.6	30.2	36.2	7.2
PUFA (g)	17.7	12.0	16.8	11.4	15.7	13.3	15.0	15.5	13.5	13.2	14.8	17.3	15.6	1.5
Sat. Fatty Acid (g)	45	22	37.3	24.0	34.8	23.3	31.9	19.5	25.2	16.9	24.5	20.7	33.1	7.7
Carbohydrate (g)	411	201	388	197	356	187	348	211	324	203	218	152	340.8	67.5
Alcohol (g)	13.9	44.9	10.6	43.6	10.4	31.9	45.8	111	80.4	136	4.2	22.1	27.6	29.8
TDF (g)	18.1	10.6	18.3	11.0	17.0	10.1	15.0	10.7	14.2	9.4	11.0	11.4	15.6	2.8
Calcium (mg)	1455	848	1273	1033	1360	1251	1176	825	811	574	700	508	1129.2	305.9
Iron (mg)	20.6	12.8	20.0	16.5	18.2	12.0	17.0	12.6	14.0	8.4	10.9	7.3	16.8	3.7
Magnesium (mg)	385	207	360	212	345	228	326	189	318	208	218	192	325.3	57.8
Phosphorous (mg)	2011	960	1791	1098	1795	1200	1675	917	1374	727	1072	723	1619.7	339.6
Potassium (mg)	4528	1920	4314	2399	3737	2141	3365	1718	3062	1568	2242	1420	3541.3	843.6
Sodium (mg)	4370	2160	4096	2419	3695	1811	3482	1710	2998	1993	2831	2314	3578.7	602.1
Zinc (mg)	19.3	11.5	15.1	12.4	15.5	10.1	13.4	8.9	11.5	7.1	9.3	6.0	14.0	3.5
Copper (mg)	2.00	1.09	1.77	1.38	1.69	1.03	1.60	0.98	1.36	0.83	1.10	0.83	1.6	0.3
Vitamin A (IU)	6296	5656	8610	9282	7112	7337	5885	6575	4032	5955	6016	14797	6325.2	1509.2
Vitamin A (RE)	1169	1089	1320	1251	1187	1006	972	941	617	659	828	1510	1015.5	261.4
Carotenes (mg)	346	410	419	681	458	676	376	587	292	605	411	1438	383.7	59.0
Vitamin C (mg)	218	231	241	208	200	208	145	180	101	97	77.7	86.9	163.8	66.2
Vitamin E (mg)	17.5	47.6	13.1	20.4	11.1	15.0	11.0	12.5	7.0	6.8	6.7	6.0	11.1	4.0
Vitamin B1 (mg)	2.83	3.64	2.62	3.57	2.71	3.40	1.92	1.26	1.69	0.92	1.29	0.90	2.2	0.6
Vitamin B2 (mg)	3.64	3.39	3.29	3.86	3.36	3.88	2.79	1.76	2.29	1.32	1.72	1.14	2.8	0.7
Vitamin B3 (mg)	33.0	20.0	28.5	19.8	28.9	20.8	29.3	18.6	25.4	16.6	18.6	13.4	27.3	4.9
Vitamin B6 (mg)	3.14	3.13	2.63	3.61	2.72	3.59	2.31	1.61	2.28	1.54	1.28	0.78	2.4	0.6
Folacin (mg)	404	246	430	251	398	326	347	253	307	223	192	155	346.3	87.6
Vitamin B12 (mcg)	8.61	5.58	6.44	6.53	6.66	6.87	5.36	3.98	4.43	3.05	4.15	3.11	5.9	1.7
Cholesterol (mg)	466	265	398	278	398	278	324	224	246	183	263	265	349.2	86.2

Table 3. Mean Percentage of Total Energy for Macronutrients

Date	% Protein	% Fat	% CHO	% Alcohol	% SFA	% MUFA	% PUFA
7/16/96	15.1	33.6	49.4	2.9	12.2	12.8	4.8
7/17/96	13.8	32.6	52.4	2.5	11.4	12.8	5.1
7/18/96	13.6	28.2	48.2	11.0	9.9	10.6	4.7
7/19/96	13.6	28.2	48.2	11.0	9.9	10.6	4.7
7/20/96	11.5	24.2	45.7	19.6	8.0	9.5	4.3
7/21/96	15.1	37.1	47.3	1.6	12.0	14.0	7.2
7/31/96	14.0	32.0	42.6	12.0	12.0	13.7	3.8
8/1/96	12.8	27.7	48.2	12.2	10.1	11.7	3.7
Total	13.7	30.3	48.4	8.6	10.7	11.8	4.8

Table 4. Average Caloric Intake by Meal, by Day and Number of Subjects

		07/16/96	07/17/96	7/18/96	7/19/96	7/20/96	7/21/96	
Breakfast	N	62	72	64	46	44	33	
	Mean	948	958	986	886	993	1139	Mean = 985
	SD	369	466	376	365	392	455	SD = 85
Lunch	N	57	47	56	60	5	2	
	Mean	1122	1089	986	1180	1107	992	Mean = 1079
	SD	423	427	376	419	932	459	SD = 76
Dinner	N	71	62	70	40	38	42	
	Mean	1171	1190	1099	1076	1262	1042	Mean = 1140
	SD	369	387	460	442	465	365	SD = 82
Snack	N	58	51	39	35	27	11	
	Mean	1098	1069	1060	2060	2343	1294	Mean = 1487
	SD	918	718	804	1944	1571	1428	SD = 567
	Day Totals	4339	4307	4131	5202	5705	4467	Average= 4692

Table 5. Distribution of Calcium Intake Among U.S. Army Rangers

	N	%
Above 1200 mg	31	33.7
800 - 1200 mg	24	26.1
700 - 799 mg	16	17.4
600 - 699 mg	7	7.6
500 - 599 mg	4	4.3
Below 500 mg	10	10.9

Table 6. Mean Percent Contributions to Mean Energy Intake for Six Days

		Protein	Fat	CHO	Kcal	Alcohol	SFA	MUFA	PUFA
7/16/96 Total	Breakfast	2394.7	2098.4	9354.9	64897.9	0	791.4	763.9	271.5
7/17/96 Total		2448.9	2417.1	9586.1	69004.3	0	847.5	906.8	325.7
7/18/96 Total		2312.6	2255.3	8362.2	62314.6	0	806.9	803.1	299.7
7/19/96 Total		1587.9	1296.3	5798.4	40737.6	0	497.0	475.6	165.0
7/20/96 Total		1636.0	1495.1	6029.0	43682.0	0	485.8	576.5	267.7
7/21/96 Total		1484.4	1651.8	4289.4	37594.2	0	567.2	597.6	276.7
Total		11864.5	11214.0	43420.0	318230.6	0	3995.9	4123.6	1606.4
% Contribution		14.9	31.7	54.6		0	11.3	11.7	4.5
7/16/96 Total	Lunch	2782.2	2521.3	6050.4	57841.3	0.0	932.9	906.4	366.5
7/17/96 Total		2194.7	2038.9	5969.9	50609.8	0.0	687.4	801.6	331.4
7/18/96 Total		2310.9	2048.1	8226.8	60090.9	0.0	668.9	717.8	382.6
7/19/96 Total		3021.7	2794.1	8530.9	70819.1	0.0	918.1	1003.7	561.5
7/20/96 Total		217.0	122.3	891.6	5532.5	2.1	49.1	48.7	14.7
7/21/96 Total		135.0	90.6	154.3	1984.4	0.0	30.6	37.7	13.5
Total		10661.6	9615.2	29824.0	246878.0	2.1	3287.0	3515.9	1670.1
% Contribution		17.3	35.1	48.3		0.0	12.0	12.8	6.1
7/16/96 Total	Dinner	3298.8	3559.4	9221.2	83112.8	190.1	1239.2	1387.5	508.4
7/17/96 Total		2687.7	2941.7	9386.8	73805.4	0.0	1004.3	1199.3	493.2
7/18/96 Total		3672.8	2870.8	9193.1	76936.3	0.0	1063.7	1089.4	471.4
7/19/96 Total		1672.0	1590.3	5524.9	43049.8	34.6	534.9	614.0	278.6
7/20/96 Total		1894.7	1811.7	5720.1	47974.2	229.0	613.1	698.6	310.5
7/21/96 Total		1716.9	1610.5	5661.5	43753.9	0.5	531.8	598.5	321.6
Total		14942.9	14384.5	44707.6	368632.5	454.1	4986.9	5587.4	2383.7
% Contribution		16.2	35.1	48.5		0.9	12.2	13.6	5.8
7/16/96 Total	Snack	1703.1	1895.3	8633.8	63705.2	938.0	698.9	772.8	287.0
7/17/96 Total		1267.2	1583.2	7655.1	55100.4	886.7	592.5	621.1	260.4
7/18/96 Total		1210.2	1249.7	5112.8	42126.1	896.3	482.6	460.9	218.3
7/19/96 Total		1427.8	1414.3	7464.2	72105.9	3540.3	547.5	584.8	180.8
7/20/96 Total		858.5	892.7	5695.6	63271.5	4270.5	279.7	373.7	176.1
7/21/96 Total		350.4	665.1	1441.9	14237.5	222.7	168.9	280.9	173.7
Total		6817.2	7700.2	36003.4	310546.6	10754.6	2770.1	3094.2	1296.4
% Contribution		8.8	22.3	46.4		24.2	8.0	9.0	3.8
Total for 6 days		44286.2	42913.9	153955.0	1244287.7	11210.7	15039.8	16321.1	6956.5
% Contribution		14.23664	31.03988	49.49178		6.306815	10.87839	11.80512	5.031694

Table 7. Milk Consumption in an Average Day as Observed in U.S. Army Rangers

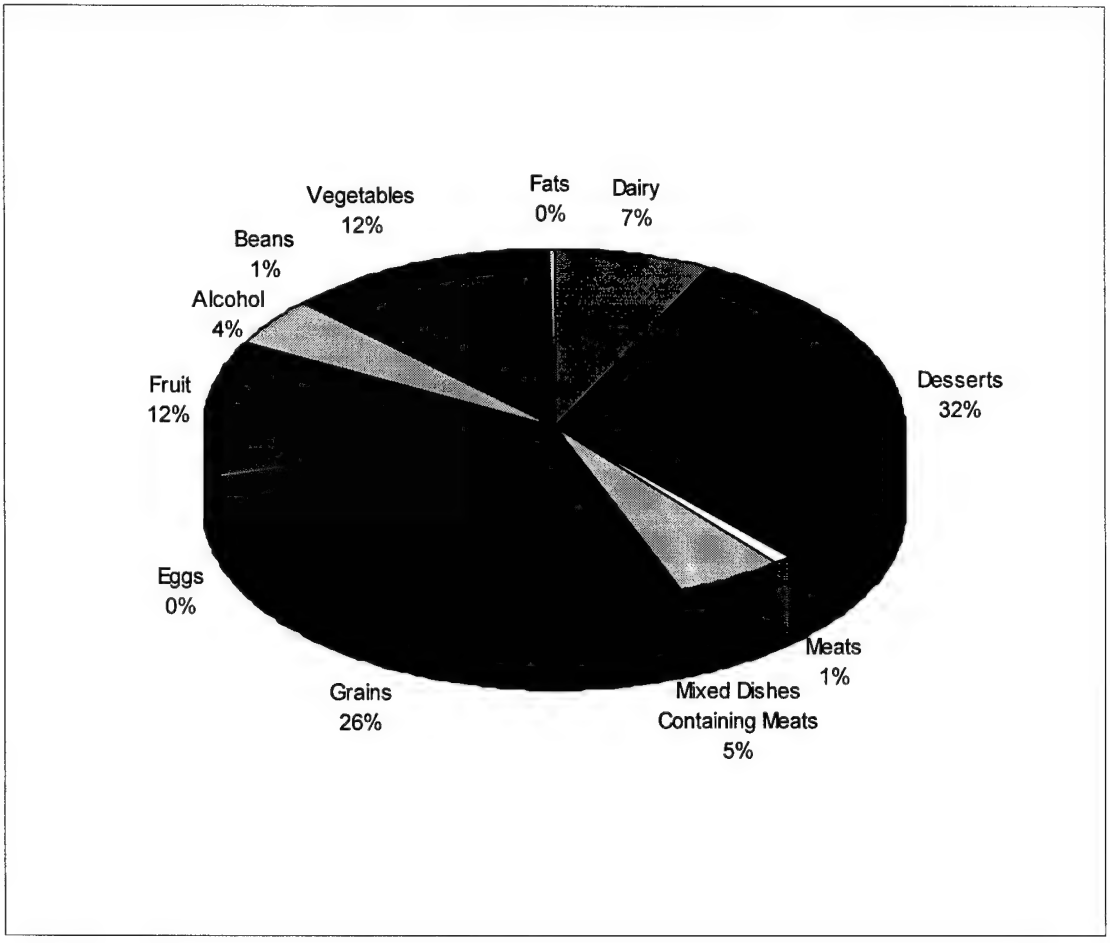
Glasses per Day													
Day	0		0 - 0.5		1		1.5		2		>2		
	N	%	N	%	N	%	N	%	N	%	N	%	Total N
1	36	44	4	4.9	11	13.6	1	1.2	9	11.1	20	24.7	81
2	39	46	3	3.6	11	13.1	1	1.2	13	15.5	17	20.2	84
3	44	51	3	3.4	7	8.0	2	2.3	7	8.0	23	26.4	87
4	45	57	2	2.5	4	5.1	4	5.1	14	17.7	10	12.7	79
5	35	61	2	3.5	8	14.0	0	0.0	8	14.0	4	7.0	57
6	32	60	2	3.8	5	9.4	0	0.0	10	18.9	4	7.5	53

Table 8. Number of Visible Eggs Eaten per Day

		number of eggs consumed							
Day	Egg Type	0	0 - 1	2	3	4	5	6	7 +
1	Whole	47	4	25	3	2	0	0	0
	White	62	0	3	7	8	1	0	0
2	Whole	51	4	23	2	3	0	1	0
	White	65	0	4	3	9	1	1	1
3	Whole	52	5	27	1	2	0	0	0
	White	70	1	4	4	6	1	1	0
4	Whole	59	1	18	0	1	0	0	0
	White	66	0	2	3	6	0	1	1
5	Whole	50	1	5	0	1	0	0	0
	White	47	0	2	2	3	1	2	0
6	Whole	41	5	5	1	0	1	0	0
	White	46	1	2	1	1	0	2	0

Percent Contributions of Major Food Groups to Total CHO Intake

	% CHO	Protein	Fat	CHO	Kcal	Alcohol
Dairy	7.2	7776.508	5711.72	11090.06	126331.2	0
Desserts	31.1	1549.629	2991.988	47675.25	217734.4	1.227065
Meats	0.9	9277.548	7863.303	1389.08	115720.2	0
Mixed Dishes Containing Meats	5.0	8838.136	7269.134	7740.339	133105.5	0
Grains	26.0	8245.998	6890.766	39888.49	255772.6	0
Eggs	0.3	2748.293	1500.348	404.9673	27014.28	0
Fruit	12.3	920.2777	235.9753	18835.87	76867.88	0
Alcohol	4.5	593.4684	38.9022	6878.264	107411.8	11207.88
Beans	0.7	872.4809	1536.111	1104.327	20433.62	0
Vegetables	11.7	3215.427	4626.143	17985.65	121633.2	0
Fats	0.3	47.42547	4239.54	472.7321	39350.03	0
Total	100.0	44085.19	42903.93	153465	1241375	11209.11

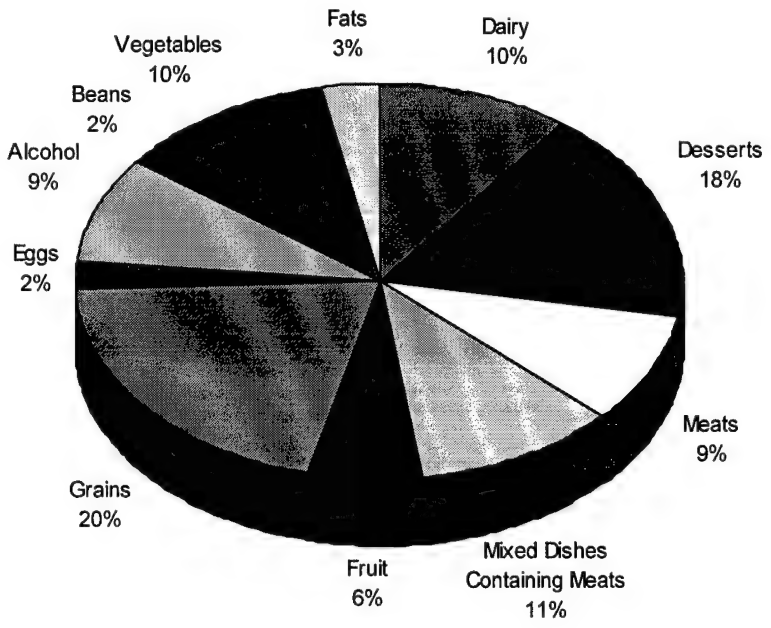


Percent Contribution of Total Calories from Dining Facility versus Meals and Snacks from Other Sources

		7/16/96	7/17/96	7/18/96	7/19/96	7/20/96	7/21/96	%Total
Ranger Dining Facility	N	81	82	86	76	54	48	
	Average	2377.7	2112.5	2026.9	1909.0	1433.0	1556.0	
	SD	951.5	1099.6	949.8	909.1	595.2	794.0	
	%	71.4	69.7	72.2	64.0	48.2	76.5	67.3
	Total	192595.4	173227.3	174311.2	145083.8	77379.7	74688.0	
Outside Food Sources	N	57	53	44	34	28	12	
	Average	1147.049	1273.53	1325.253	1395.65	1430.746	1740.629	
	SD	951.9141	817.3001	962.6721	1240.258	1034.15	1367.055	
	%	24.25525	27.15963	24.14862	20.93051	24.96624	21.40776	24.07727
	Total	65381.77	67497.09	58311.14	47452.09	40060.89	20887.55	
Alcohol	N	13	9	11	22	23	2	
	Average	890.7692	866.1778	804.134	1553.483	1870.423	997.2	
	SD	898.5378	704.1606	430.5227	1376.384	1302.678	300.379	
	%	4.295935	3.13681	3.66321	15.07488	26.8102	2.044072	8.632395
	Total	11580	7795.6	8845.474	34176.63	43019.72	1994.4	

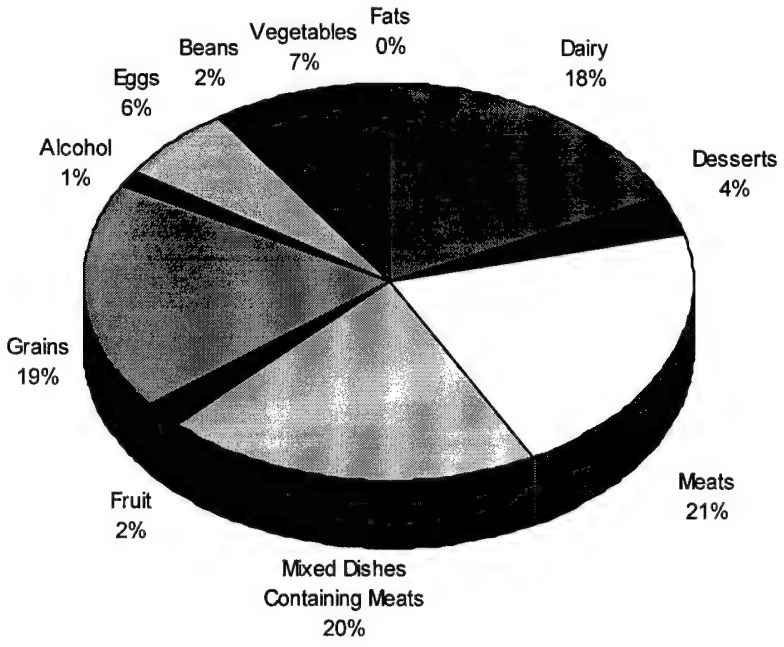
Percent Contributions of Major Food Groups to Total Energy Intake

	% Kcal	Protein	Fat	CHO	Kcal	Alcohol
Dairy	10.2	7776.508	5711.72	11090.06	126331.2	0
Desserts	17.5	1549.629	2991.988	47675.25	217734.4	1.227065
Meats	9.3	9277.548	7863.303	1389.08	115720.2	0
Mixed Dishes Containing Meats	10.7	8838.136	7269.134	7740.339	133105.5	0
Fruit	6.2	920.2777	235.9753	18835.87	76867.88	0
Grains	20.6	8245.998	6890.766	39888.49	255772.6	0
Eggs	2.2	2748.293	1500.348	404.9673	27014.28	0
Alcohol	8.7	593.4684	38.9022	6878.264	107411.8	11207.88
Beans	1.6	872.4809	1536.111	1104.327	20433.62	0
Vegetables	9.8	3215.427	4626.143	17985.65	121633.2	0
Fats	3.2	47.42547	4239.54	472.7321	39350.03	0
Total	100.0	44085.19	42903.93	153465	1241375	11209.11

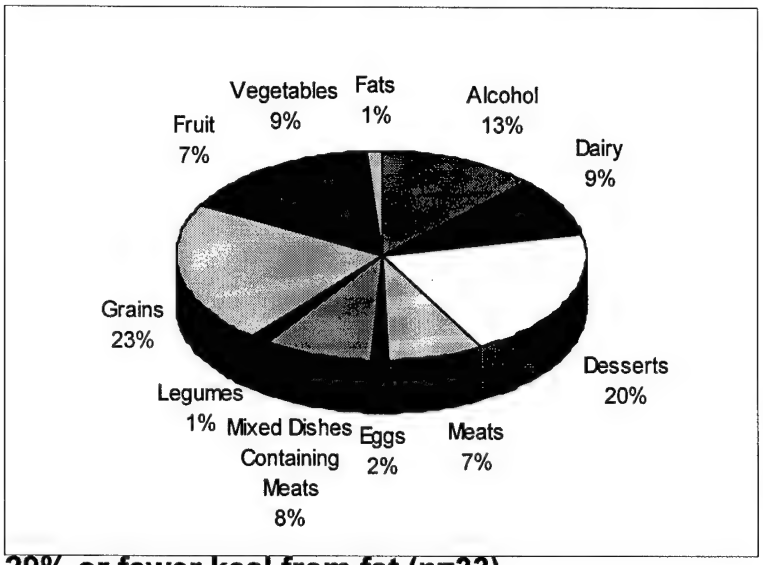


Percent Contributions of Major Food Groups to Total Protein Intake

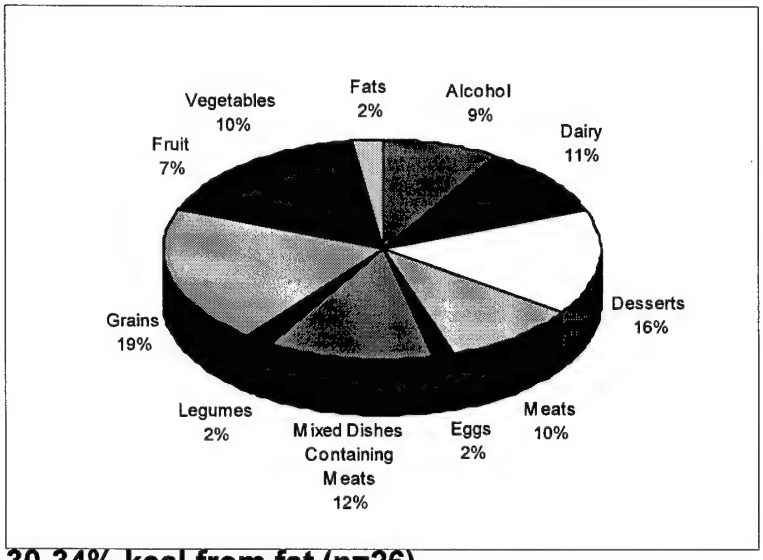
	% Protein	Protein	Fat	CHO	Kcal	Alcohol
Dairy	17.6	7776.508	5711.72	11090.06	126331.2	0
Desserts	3.5	1549.629	2991.988	47675.25	217734.4	1.227065
Meats	21.0	9277.548	7863.303	1389.08	115720.2	0
Mixed Dishes Containing Meats	20.0	8838.136	7269.134	7740.339	133105.5	0
Fruit	2.1	920.2777	235.9753	18835.87	76867.88	0
Grains	18.7	8245.998	6890.766	39888.49	255772.6	0
Alcohol	1.3	593.4684	38.9022	6878.264	107411.8	11207.88
Eggs	6.2	2748.293	1500.348	404.9673	27014.28	0
Beans	2.0	872.4809	1536.111	1104.327	20433.62	0
Vegetables	7.3	3215.427	4626.143	17985.65	121633.2	0
Fats	0.1	47.42547	4239.54	472.7321	39350.03	0
Total	100.0	44085.19	42903.93	153465	1241375	11209.11



Percent Contribution of Major Food Groups at Different Fat Intake Levels of U.S. Army Rangers

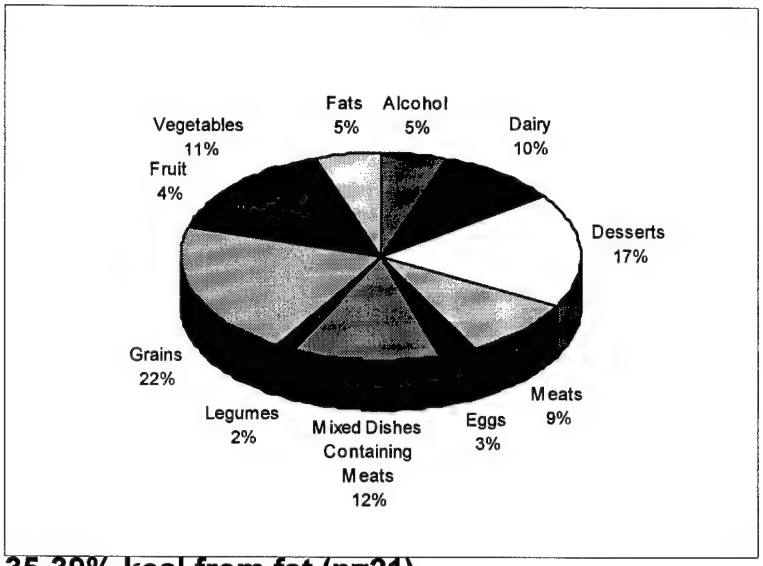


29% or fewer kcal from fat (n=33)

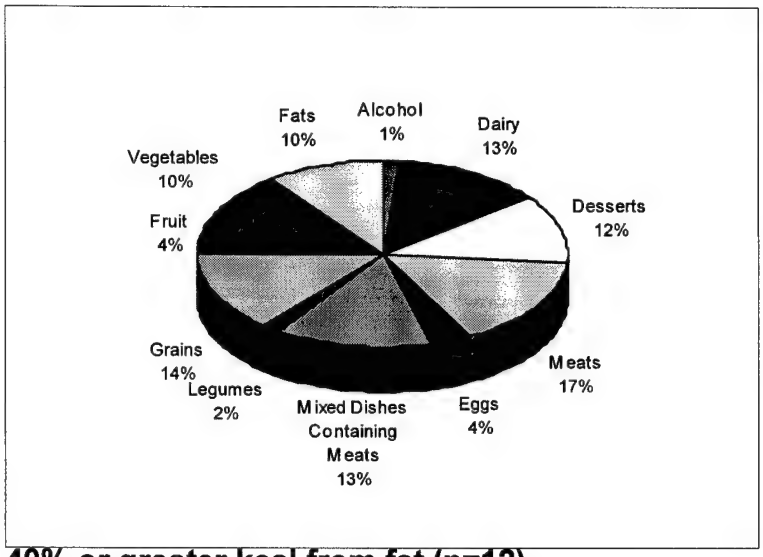


30-34% kcal from fat (n=26)

Percent Contribution of Major Food Groups at Different Fat Intake Levels of U.S. Army Rangers (continued)



35-39% kcal from fat (n=21)



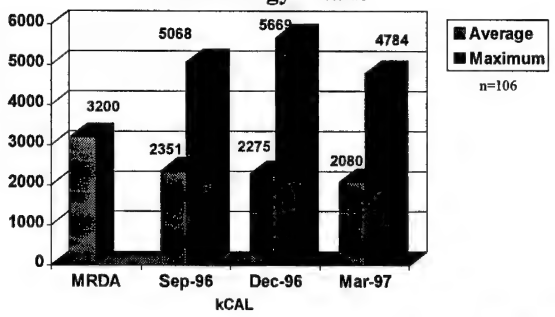
40% or greater kcal from fat (n=12)

Distribution of Dietary Cholesterol Intakes at Breakfast, Lunch, Dinner, and Snacks

		16-Jul	17-Jul	18-Jul	19-Jul	20-Jul	21-Jul	31-Jul	1-Aug	
Breakfast	N	62	72	64	46	44	33	14	23	
	Mean	224.9	230.6	262.1	229.7	162.6	242.0	250.0	285.4	Mean = 235.9
	SD	225.7	261.1	233.5	244.8	199.4	241.3	193.1	293.9	SD = 35.7
Lunch	N	57	47	56	60	5	2	21	27	
	Mean	153.3	128.4	87.2	136.3	68.8	169.0	95.1	98.3	Mean = 117.0
	SD	80.7	83.6	59.0	55.9	76.3	13.6	61.4	49.8	SD = 35.0
Dinner	N	71	62	70	40	38	42	28	27	
	Mean	150.6	120.1	136.5	97.0	144.6	107.8	136.4	134.6	Mean = 128.4
	SD	92.0	89.2	83.9	66.1	79.8	82.2	111.8	94.8	SD = 18.5
Snack	N	58	51	39	35	27	11	26	28	
	Mean	76.0	60.6	82.2	78.1	45.4	97.1	91.8	54.7	Mean = 73.2
	SD	105.2	87.8	170.5	130.4	59.3	175.2	123.9	97.2	SD = 18.2
	Day Totals	604.8	539.6	568.1	541.1	421.3	615.9	573.3	573.0	Avg= 554.6

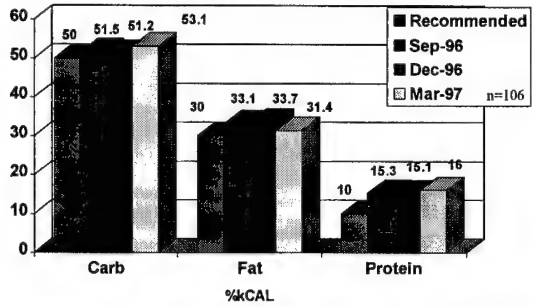
RESULTS

Total Energy Intake



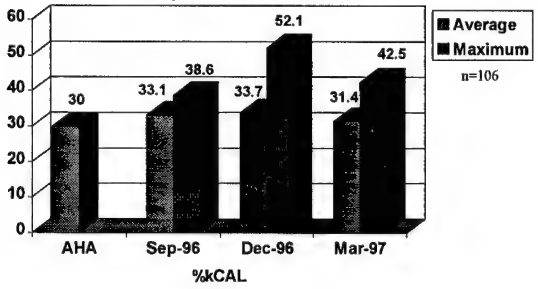
RESULTS

Energy Intake Distribution



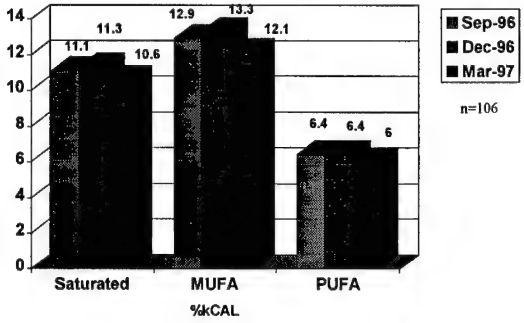
RESULTS

Dietary Total Fat Intake



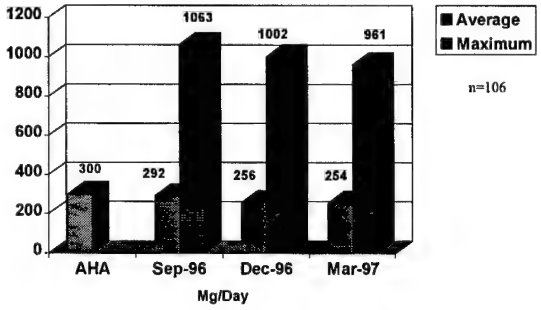
RESULTS

Dietary Fat Intake



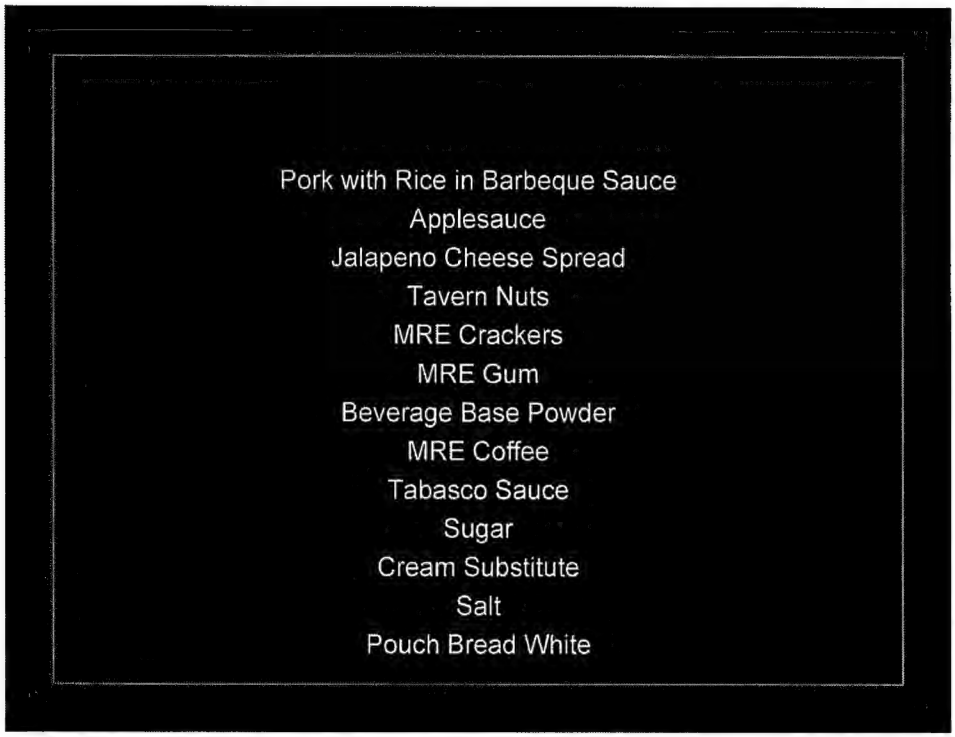
RESULTS

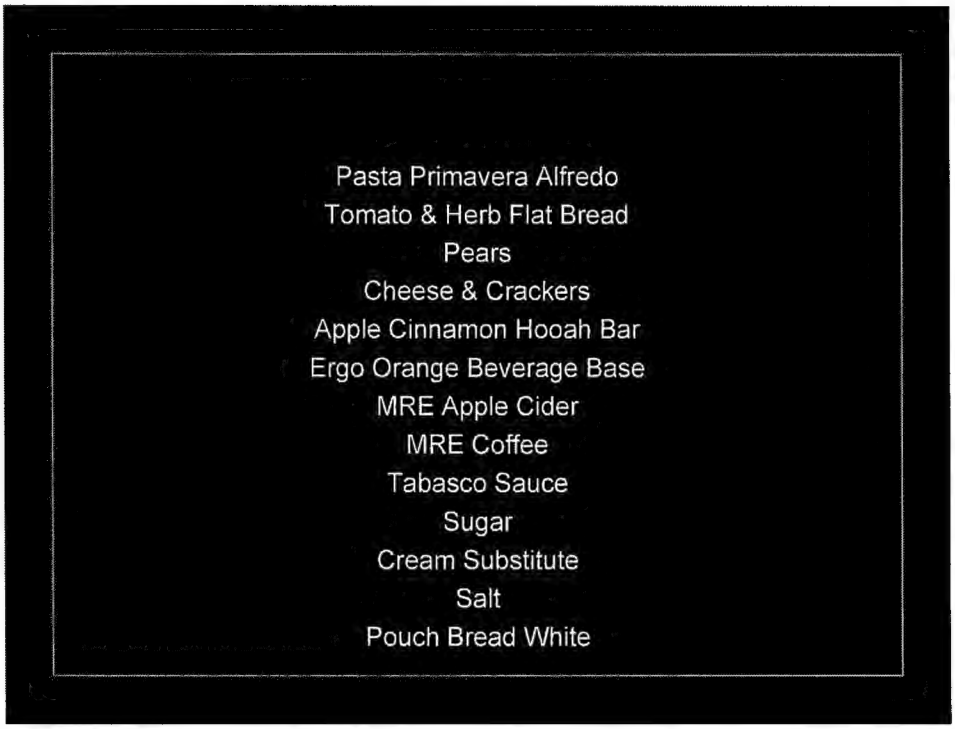
Dietary Cholesterol Intake



CONCLUSIONS

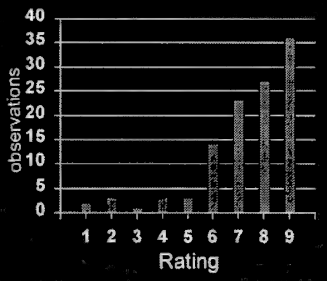
- **The average diet for USASMA students approaches dietary recommendations.**
- **There was no significant change in the average diet over time.**
- **Serum lipid levels increased over time.**

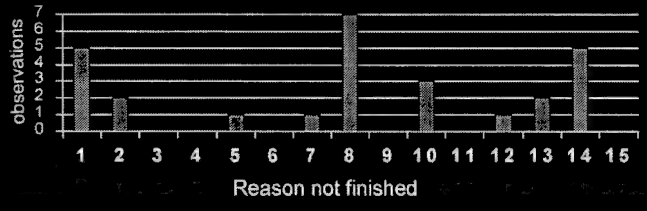

Pork with Rice in Barbeque Sauce
Applesauce
Jalapeno Cheese Spread
Tavern Nuts
MRE Crackers
MRE Gum
Beverage Base Powder
MRE Coffee
Tabasco Sauce
Sugar
Cream Substitute
Salt
Pouch Bread White

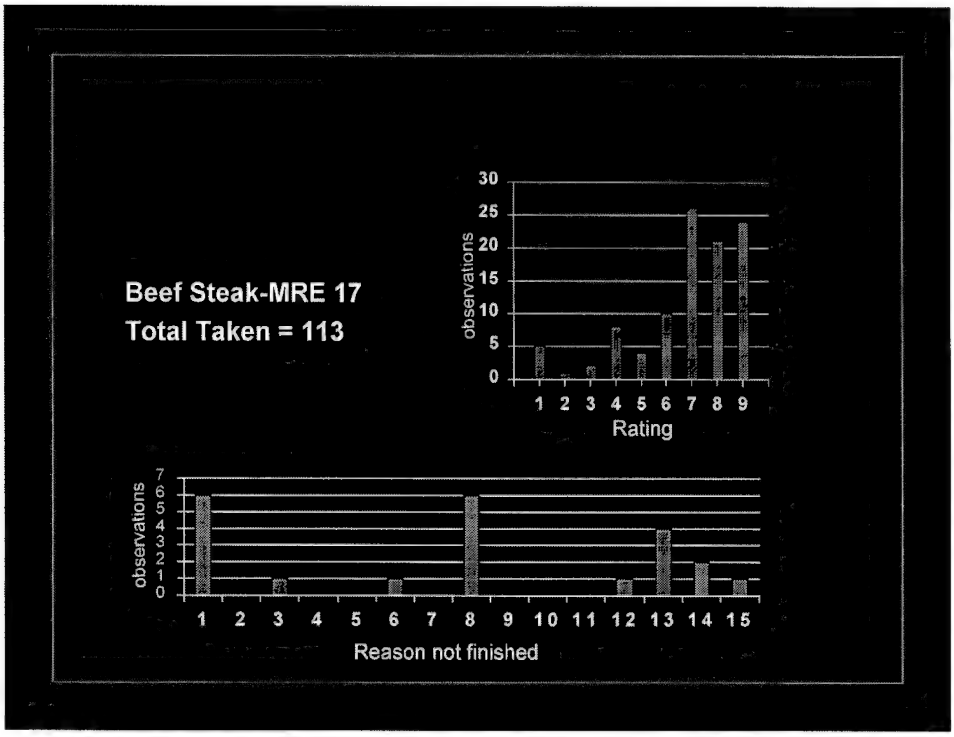

Pasta Primavera Alfredo
Tomato & Herb Flat Bread
Pears
Cheese & Crackers
Apple Cinnamon Hooah Bar
Ergo Orange Beverage Base
MRE Apple Cider
MRE Coffee
Tabasco Sauce
Sugar
Cream Substitute
Salt
Pouch Bread White

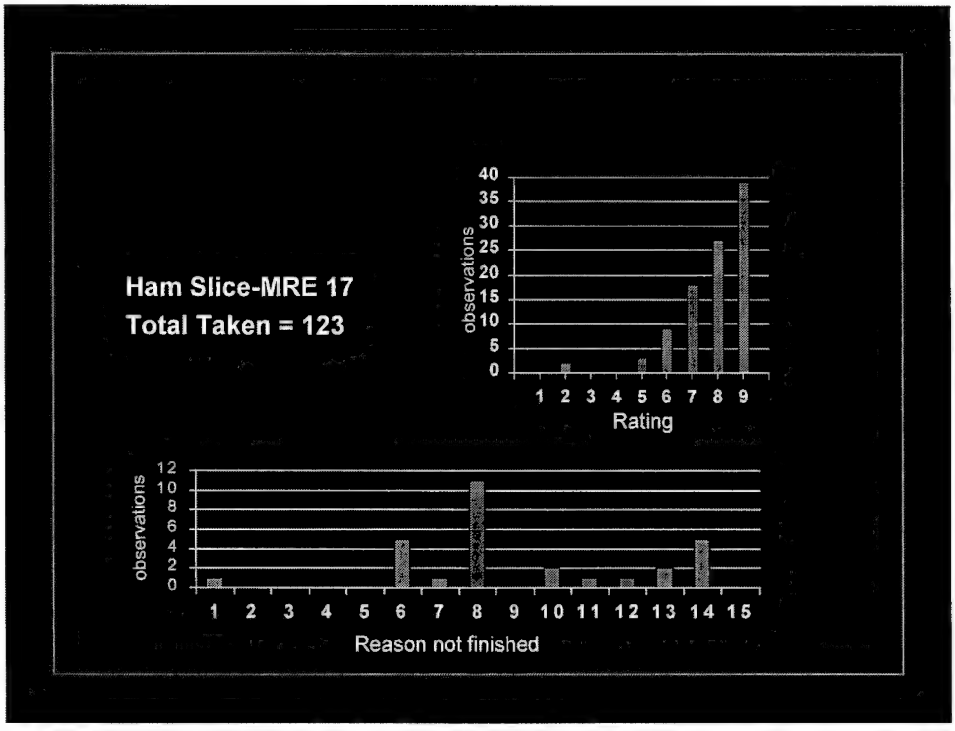
1.	Taste	9.	No Water
2.	Smell	10.	No Time
3.	Texture	11.	Damaged
4.	Looks	12.	Ill/Sick
5.	Dieting	13.	Other
6.	Full	14.	Not Hungry
7.	Traded	15.	Inappropriate Food/ Water Temperature
8.	Saved		

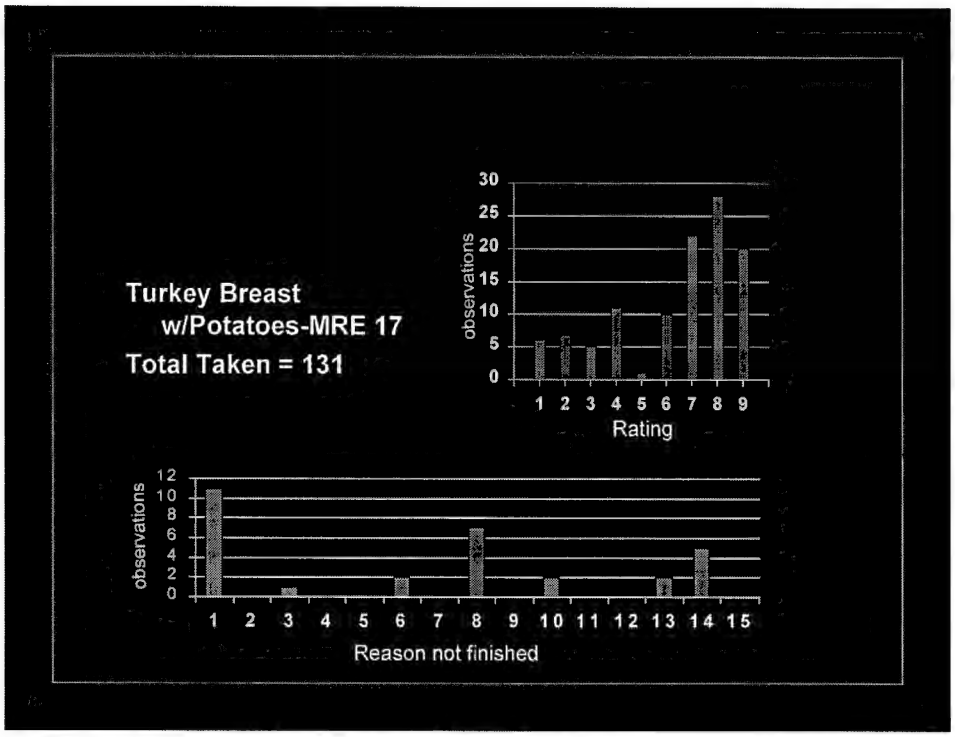
Pork w/Rice-MRE 17
Total Taken = 132

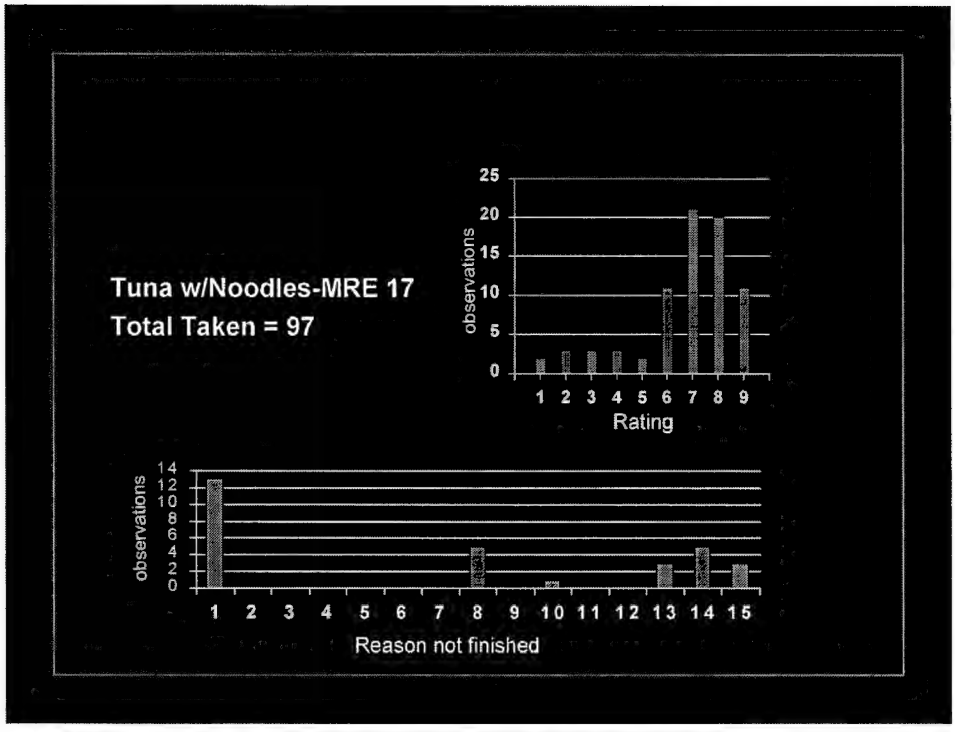


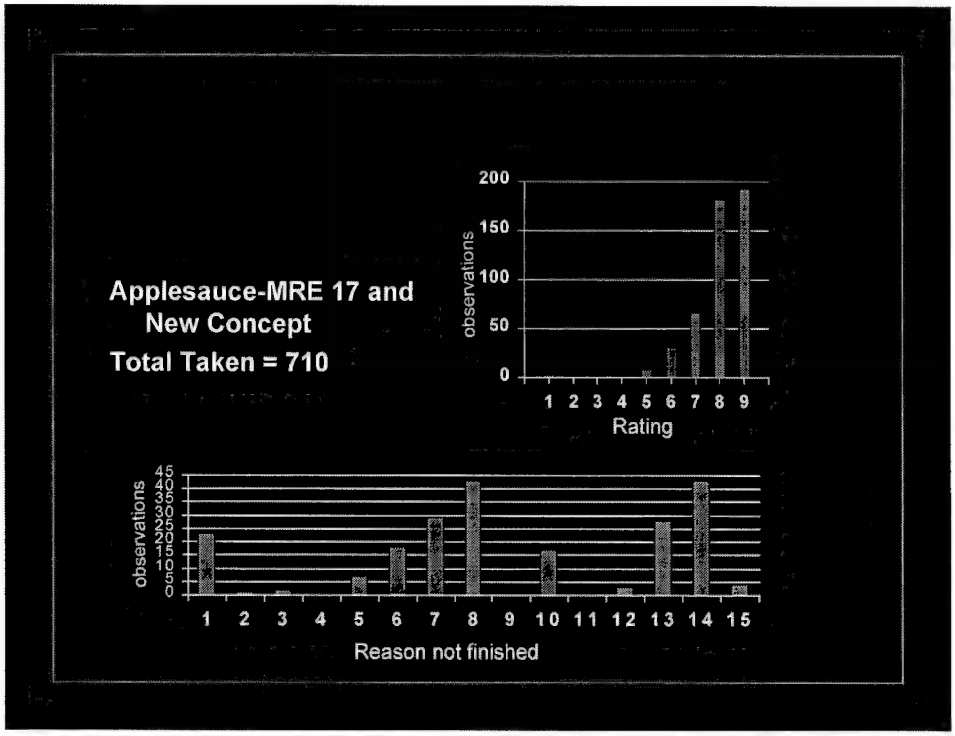



Beef Steak-MRE 17
Total Taken = 113
observations
Rating
Reason not finished


Ham Slice-MRE 17
Total Taken = 123
observations
Rating
Reason not finished


Turkey Breast
w/Potatoes-MRE 17
Total Taken = 131
observations
Rating
Reason not finished


Tuna w/Noodles-MRE 17
Total Taken = 97
observations
Rating
Reason not finished


Applesauce-MRE 17 and
New Concept
Total Taken = 710
observations
200
150
100
50
0
1 2 3 4 5 6 7 8 9
Rating
observations
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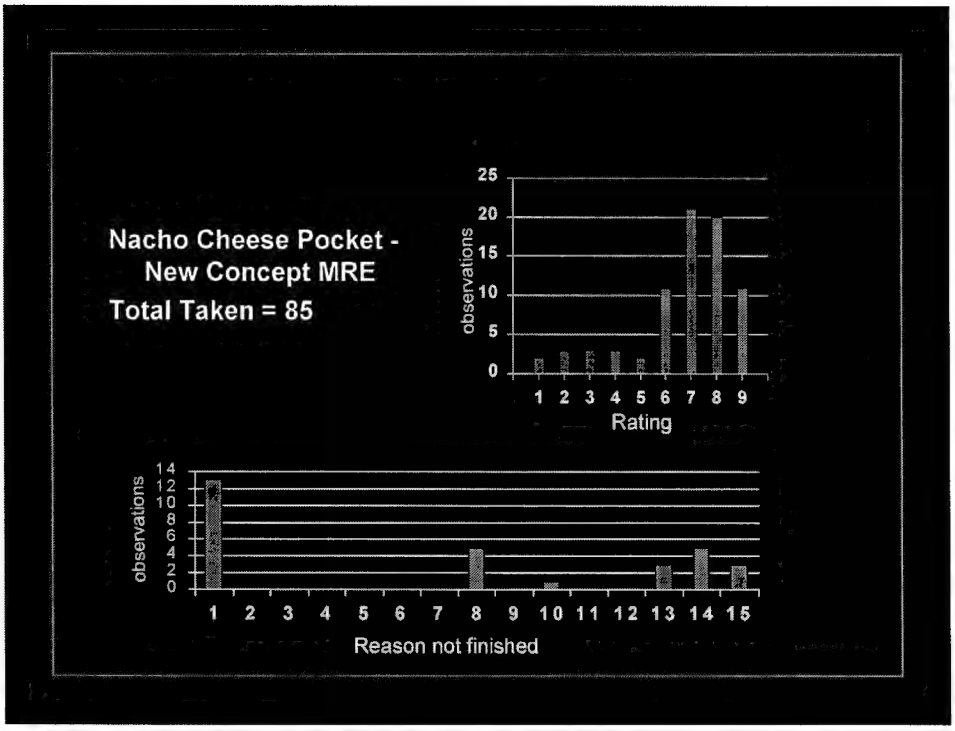
40
35
30
25
20
15
10
5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Reason not finished


Nacho Cheese Pocket -
New Concept MRE
Total Taken = 85
observations
25
20
15
10
5
0
1 2 3 4 5 6 7 8 9
Rating
observations
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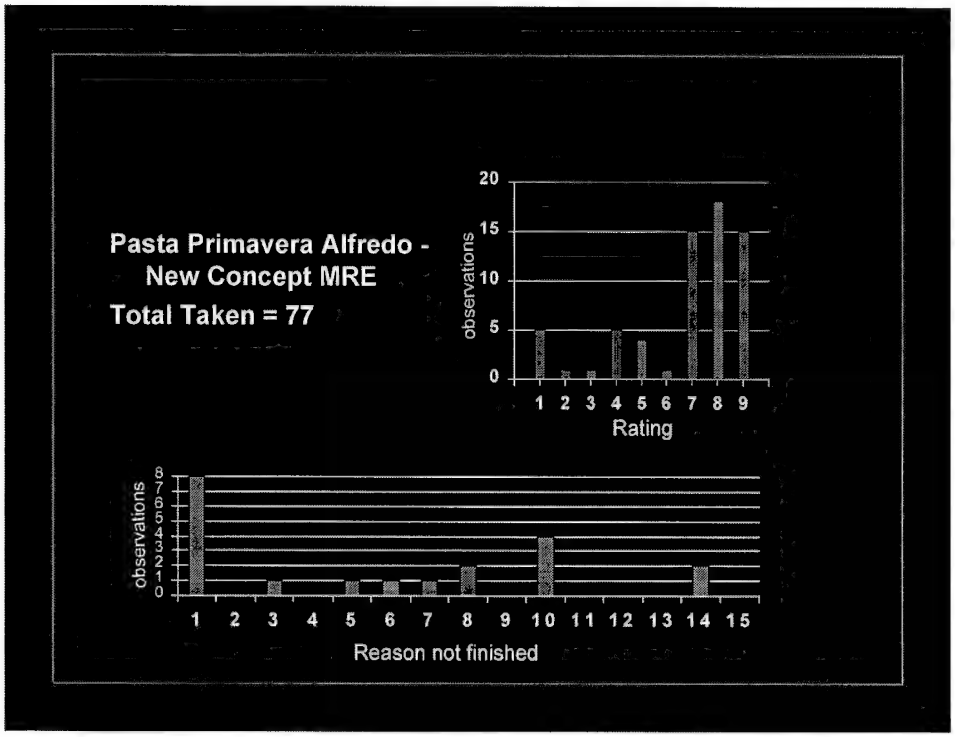
12
10
8
6
4
2
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Reason not finished


Pasta Primavera Alfredo -
New Concept MRE
Total Taken = 77
observations
20
15
10
5
0
1 2 3 4 5 6 7 8 9
Rating
observations
8
7
6
5
4
3
2
1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Reason not finished

APPENDIX
TASK 6:
ENHANCING MILITARY DIETS

Table 1.
Description of Military Respondents to Food Preference Survey by Gender (n=662)

Category	Male	Female
Gender	408	252
Age (years)		
18-21	227	194
22-25	103	40
26-29	43	8
30 and over	34	8
Ethnic Origin		
Caucasian	220	132
African American	83	67
Asian	11	5
Hispanic	51	26
Am. Indian	8	1
Other	35	21
Geographic Breakdown		
Middle Atlantic	64	35
East North Central	49	31
East South Central	12	15
West South Central	70	39
Pacific	42	31
New England	22	16
West North Central	22	19
Atlantic	80	41
Mountain	18	8
Other	19	9

Table 2.
Gender Differences in Frequency of Selection of Specific Food Categories (n=662)

Food Category	Male (N)	%	Female (N)	%
Fast/convenience				
Once a week	166	41.3	101	40.7
3-4 times/week	160	39.8	95	38.3
once a day	30	7.5	13	5.2
twice a day	18	4.5	12	4.8
none of the above	28	7.0	27	10.9
Diet/low calorie				
Once a week	108	27.1	47	19.0
3-4 times/week	83	20.8	65	26.2
once a day	49	12.3	45	18.1
twice a day	25	6.3	37	14.9
none of the above	134	33.6	54	21.8
Heart healthy				
Once a week	87	21.6	54	22.0
3-4 times/week	120	29.9	66	26.8
once a day	82	20.4	38	15.4
twice a day	56	13.9	49	19.9
none of the above	57	14.2	39	15.9
Power/energy				
Once a week	68	16.9	59	23.8
3-4 times/week	107	26.6	51	20.6
once a day	86	21.4	37	14.9
twice a day	45	11.2	19	7.7
none of the above	96	23.9	82	33.1

Table 3.
Comparisons Between Hedonic Ratings of Foods Tested by Consumer Panels at Two Sites

Food Item	PBRC Overall	LAT Overall	PBRC 18-21 yr	LAT 18-21 yr
Chicken & Okra Gumbo	8.0 ± 0.8	6.9 ± 1.4	7.8 ± 1.3	6.9 ± 1.2
Chicken & Broccoli Pasta	7.6 ± 1.0	6.2 ± 1.9	7.4 ± 0.9	6.2 ± 1.7
French Country Stew	8.2 ± 0.8	7.7 ± 1.2	8.5 ± 0.7	8.3 ± 0.7
Homestyle Granola	7.6 ± 1.1	7.8 ± 1.3	7.6 ± 0.8	8.3 ± 0.6
Key Lime Pie	7.6 ± 1.3	6.7 ± 2.0	7.1 ± 1.0	7.1 ± 1.7
Mambo Pork Roast	7.3 ± 1.2	6.6 ± 1.5	6.8 ± 1.3	6.5 ± 1.5
Quesadillas	7.9 ± 1.0	7.5 ± 1.3	7.9 ± 0.7	7.6 ± 1.4
Roasted Pepper Potatoes	7.6 ± 1.0	6.7 ± 1.7	7.0 ± 1.6	6.6 ± 1.8
Shepherd's Pie	7.0 ± 1.4	7.0 ± 1.3	6.3 ± 1.8	7.2 ± 1.4
Strawberry Shortcake Pie	7.2 ± 1.3	7.5 ± 1.1	7.9 ± 1.1	7.7 ± 0.8

Pennington Biomedical Research Center
Louisiana State University
Food Preference Questionnaire

Instructions

Your response to this survey will help menu planners and dietitians provide the foods you want to eat on ration menus. **THIS IS NOT A TEST.** We are interested in your opinions and yours alone, so please do not compare your answers with anyone while the survey is in progress.

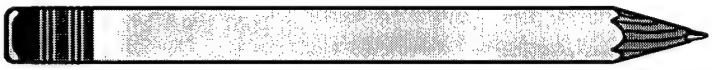
Please make sure that you **fill each bubble in fully** so that your response may be processed accurately. Check marks, lines, or slashes will not register with the scanning program.

Incorrect Marks:

☐ ⊘ ☒

Correct Mark:

■ ⊙



1. Today's Date

	DAY		YEAR
☐ Jan			
☐ Feb			
☐ Mar	0 ☐	0 ☐	☐ 97
☐ Apr	1 ☐	1 ☐	☐ 98
☐ May	2 ☐	2 ☐	☐ 99
☐ Jun	3 ☐	3 ☐	☐ 00
☐ Jul		4 ☐	
☐ Aug		5 ☐	
☐ Sep		6 ☐	
☐ Oct		7 ☐	
☐ Nov		8 ☐	
☐ Dec		9 ☐	

2. What is your rank?

- ☐ E1
- ☐ E2
- ☐ E3
- ☐ E4
- ☐ E5
- ☐ E6
- ☐ E7
- ☐ E8
- ☐ E9
- ☐ O1
- ☐ O2
- ☐ O3
- ☐ O4
- ☐ O5
- ☐ O6
- ☐ O7
- ☐ O8
- ☐ O9
- ☐ WO1
- ☐ WO2
- ☐ WO3
- ☐ WO4

3. What is your sex?

- ☐ Male
- ☐ Female

4. What is your age?

- ☐ 18-21
- ☐ 22-25
- ☐ 26-29
- ☐ 30 and over

5. What is your ethnic origin?

- ☐ Caucasian
- ☐ African American
- ☐ Asian
- ☐ Hispanic/Mexican American
- ☐ American Indian
- ☐ Other (please specify) _____

6. Where have you lived the longest? (Please mark only one.)

- ☐ Middle Atlantic (NY, NJ, PA)
- ☐ East North Central (OH, IN, IL, MI,WI)
- ☐ East South Central (KY, TN, AL, MS)
- ☐ West South Central (AR, LA, OK, TX)
- ☐ Pacific (WA, OR, CA, AK, HI)
- ☐ New England (ME, NH, MA, RI, CT)
- ☐ West North Central (MN, IA, MO, ND,SD, NE, KS)
- ☐ Atlantic (DE, MD, DC, VA, WV, NC, SC, GA, FL)
- ☐ Mountain (MT, ID, WY, CO, NM, AZ, UT, NV)
- ☐ Other Territories, Possessions or Countries

7. Rate how well you like the following cooking or specialty foods. To say how much you like or dislike the foods, look at the rating scale below.

1	2	3	4	5	6	7	8	9
Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Food Category	1	2	3	4	5	6	7	8	9
Chinese	☐	☐	☐	☐	☐	☐	☐	☐	☐
Mexican	☐	☐	☐	☐	☐	☐	☐	☐	☐
Italian/Spanish	☐	☐	☐	☐	☐	☐	☐	☐	☐
Japanese	☐	☐	☐	☐	☐	☐	☐	☐	☐
Eastern European/German/Polish/Russian	☐	☐	☐	☐	☐	☐	☐	☐	☐
Caribbean	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cajun/Creole	☐	☐	☐	☐	☐	☐	☐	☐	☐
Far Eastern/Vietnamese/Thai	☐	☐	☐	☐	☐	☐	☐	☐	☐
French	☐	☐	☐	☐	☐	☐	☐	☐	☐
Southern/Soul	☐	☐	☐	☐	☐	☐	☐	☐	☐
Mediterranean/Greek/Middle Eastern	☐	☐	☐	☐	☐	☐	☐	☐	☐
Indian	☐	☐	☐	☐	☐	☐	☐	☐	☐
Vegetarian	☐	☐	☐	☐	☐	☐	☐	☐	☐
General American Style	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other (write in) _____	☐	☐	☐	☐	☐	☐	☐	☐	☐

8. How often do you eat "Fast Foods" or "Convenience Foods"?

- ☐ Once a week
- ☐ 3-4 times a week
- ☐ Once a day
- ☐ Twice a day
- ☐ None of the above

9. How often do you eat Low Calorie/ Diet Food?

- ☐ Once a week
- ☐ 3-4 times a week
- ☐ Once a day
- ☐ Twice a day
- ☐ None of the above

10. How often do you eat Heart Healthy/ Low Cholesterol Food?

- ☐ Once a week
- ☐ 3-4 times a week
- ☐ Once a day
- ☐ Twice a day
- ☐ None of the above

11. How often do you eat "Power" or Energy Food?

- ☐ Once a week
- ☐ 3-4 times a week
- ☐ Once a day
- ☐ Twice a day
- ☐ None of the above

INSTRUCTIONS

On this section you will be asked how much you like or dislike eating various foods. Please consider each food in a general way rather than any particular time you have eaten it.

To answer this section, proceed as follows:

4. Look at the food name and decide whether you are familiar with or have tried it.
5. If you are not familiar with or have never tried the food, darken the oval in the Never Tried column and go on to the next food.
6. If you are familiar with or have tried the food, decide how much you like or dislike it and fill in one oval under "how much you like or dislike the food" heading. To say how much you like or dislike a food, look at the rating scale below.

1	2	3	4	5	6	7	8	9
Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Food Item	Never Tried	1	2	3	4	5	6	7	8	9
Hamburger	▢	▢	▢	▢	▢	▢	▢	▢	▢	▢
Hash Browned Potatoes	▢	▢	▢	▢	▢	▢	▢	▢	▢	▢
Herb Roasted Chicken	▢	▢	▢	▢	▢	▢	▢	▢	▢	▢
Honey Baked Ham	▢	▢	▢	▢	▢	▢	▢	▢	▢	▢
Lasagna	▢	▢	▢	▢	▢	▢	▢	▢	▢	▢
Lemon Meringue Pie	▢	▢	▢	▢	▢	▢	▢	▢	▢	▢
Meatless Beans over Rice	▢	▢	▢	▢	▢	▢	▢	▢	▢	▢
Minestrone	▢	▢	▢	▢	▢	▢	▢	▢	▢	▢
Oatmeal	▢	▢	▢	▢	▢	▢	▢	▢	▢	▢
Oriental Beef Stir Fry	▢	▢	▢	▢	▢	▢	▢	▢	▢	▢
Pancakes	▢	▢	▢	▢	▢	▢	▢	▢	▢	▢
Pasta with Tomato Sauce	▢	▢	▢	▢	▢	▢	▢	▢	▢	▢
Peach Cobbler	▢	▢	▢	▢	▢	▢	▢	▢	▢	▢
Potato Salad	▢	▢	▢	▢	▢	▢	▢	▢	▢	▢
Pudding	▢	▢	▢	▢	▢	▢	▢	▢	▢	▢
Roast Beef	▢	▢	▢	▢	▢	▢	▢	▢	▢	▢
Scalloped Potatoes	▢	▢	▢	▢	▢	▢	▢	▢	▢	▢
Scrambled Eggs	▢	▢	▢	▢	▢	▢	▢	▢	▢	▢
Smothered Chicken and Rice	▢	▢	▢	▢	▢	▢	▢	▢	▢	▢
Southern Fried Chicken	▢	▢	▢	▢	▢	▢	▢	▢	▢	▢
Southern Style Greens	▢	▢	▢	▢	▢	▢	▢	▢	▢	▢
Spaghetti with Meat Sauce	▢	▢	▢	▢	▢	▢	▢	▢	▢	▢
Spicy Buffalo Wings	▢	▢	▢	▢	▢	▢	▢	▢	▢	▢
Spicy Mexican Chili	▢	▢	▢	▢	▢	▢	▢	▢	▢	▢
Spicy Spanish Rice	▢	▢	▢	▢	▢	▢	▢	▢	▢	▢
Southwestern Taco Salad	▢	▢	▢	▢	▢	▢	▢	▢	▢	▢
Spinach and Artichoke Dip	▢	▢	▢	▢	▢	▢	▢	▢	▢	▢
Steamed Vegetables	▢	▢	▢	▢	▢	▢	▢	▢	▢	▢
Stir Fried Rice	▢	▢	▢	▢	▢	▢	▢	▢	▢	▢
Stuffed Pork Roast	▢	▢	▢	▢	▢	▢	▢	▢	▢	▢

1	2	3	4	5	6	7	8	9
Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Food Item	Never Tried	1	2	3	4	5	6	7	8	9
Tuna Casserole	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Veggie Burger	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Apple Pie	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Bagels with Cream Cheese	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Baked Potato	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Baked Stuffed Flounder	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Barbecued Ribs	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Beef and Potato Stew	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Breakfast Muffins	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Broccoli and Cheese Stuffed Baked Potato	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Broiled Lemon Fish	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cake with Frosting	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Chicken Fried Steak with Gravy	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Chicken Gumbo	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Chicken Noodle Soup	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Chicken Pasta Salad	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Chicken Quesadillas	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Chocolate Chip Cookies	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Clam Chowder	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Creamed Beef on Toast	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Creole Snapper in Spicy Tomato Sauce	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fettuccini Alfredo	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
French Fries	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fried Fish Fillets	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fried Okra	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fried Pork Chops	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fruit Salad	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Garden Vegetable Lasagna	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Granola	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Greek Vegetarian Pasta	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Green Bean Casserole	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Green Salad with Dressing	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Pennington Biomedical Research Center
Louisiana State University

Food Preparation Questionnaire

INSTRUCTIONS

Your response to this survey will help recipe developers in determining the best food preparation techniques for new recipes being developed and tested for the Armed Forces Recipe File.

Read each question carefully. Mark your answer by filling in the square(s) beside the correct answer. Some of the questions will ask you to fill in more than one answer.

1. How long have you been in the military?
   - ☐ 0-2 years
   - ☐ 3-5 years
   - ☐ 6-10 years
   - ☐ 11-15 years
   - ☐ 16-20 years
   - ☐ more than 20 years
   - ☐ N/A – I am a civilian

2. How long have you been in your specialty? (If you are a civilian, how long have you been employed as a cook?)
   - ☐ 0-6 months
   - ☐ 7 months-1 year
   - ☐ 1-2 years
   - ☐ 2-3 years
   - ☐ over 3 years

3. Which of the following food service **cooking** equipment do you **have** in your kitchen or galley? (Choose **all** that apply)
   - ☐ Convection Ovens
   - ☐ Conveyer Oven (Conveyor Belt Oven)
   - ☐ Flat Top Griddle
   - ☐ Microwave Ovens
   - ☐ Steamers
   - ☐ Tilt Griddle (or Braiser)
   - ☐ Conventional Ovens
   - ☐ Deck Ovens (Pizza Deck Oven)
   - ☐ Hot Water
   - ☐ Steam Kettle
   - ☐ Stove with Burners
   - ☐ Other _____

4. Is the majority of your Food Service Cooking Equipment powered by gas or electricity?
   - ☐ Gas
   - ☐ Electric

5. Check **FIVE (5)** of the following Food Service **Cooking Equipment** which you primarily **prefer** to use?
   - ☐ Coal or Wood Burning Oven (Brick Oven)
   - ☐ Conventional Ovens
   - ☐ Deck Ovens (Pizza Deck Oven)
   - ☐ Gas, Coal, or Wood Burning Grill
   - ☐ Salamander (Broilers)
   - ☐ Steamers
   - ☐ Convection Ovens
   - ☐ Conveyer Oven (Conveyor Belt Oven)
   - ☐ Flat Top Griddle
   - ☐ Microwave Ovens
   - ☐ Steam Kettle
   - ☐ Stove with Burners
   - ☐ Other _____

6. Which Food **Preparation** Equipment do you **have** in your kitchen or galley? (Choose **all** that apply)
   - ☐ Apple Corer
   - ☐ Buffalo Chopper
   - ☐ Citrus Wedger (or Lemon Wedger)
   - ☐ Food Processor
   - ☐ Knives
   - ☐ Mixer
   - ☐ Potato Cutter (French Fry Cutter)
   - ☐ Rotary Apple or Potato Peelers
   - ☐ Sharpening Stone
   - ☐ Blender
   - ☐ Cherry or Olive Pitter
   - ☐ Cutting Boards
   - ☐ Juicer
   - ☐ Meat Grinder
   - ☐ Pasta Machine
   - ☐ Potato Peeler (Vegetable Peeler)
   - ☐ Sharpening Steel
   - ☐ Slicer
   - ☐ Other _____

7. Name **FIVE (5)** pieces of **preparation equipment** you most often use?
   - ☐ Apple Corer
   - ☐ Buffalo Chopper
   - ☐ Citrus Wedger (or Lemon Wedger)
   - ☐ Food Processor
   - ☐ Knives
   - ☐ Mixer
   - ☐ Potato Cutter (French Fry Cutter)
   - ☐ Rotary Apple or Potato Peelers
   - ☐ Sharpening Stone
   - ☐ Blender
   - ☐ Cherry or Olive Pitter
   - ☐ Cutting Boards
   - ☐ Juicer
   - ☐ Meat Grinder
   - ☐ Pasta Machine
   - ☐ Potato Peeler (Vegetable Peeler)
   - ☐ Sharpening Steal
   - ☐ Slicer
   - ☐ Other _____

8. Are the knives in the kitchen or galley in good condition?
   - ☐ Yes
   - ☐ No

9. Is there enough variety of knives in the kitchen or galley?
   - ☐ Yes
   - ☐ No

10. Which food **holding equipment** is used in your kitchen or galley? (Choose **all** that apply)

- ☐ Food Warmers
- ☐ Ovens
- ☐ Steamers
- ☐ Heat Lamp
- ☐ Steam Table
- ☐ Thermal Warmers (Camcariers)
- ☐ Other _____

11. What equipment do you most often use to **reheat** foods? (Choose **all** that apply)

- ☐ Convection Ovens
- ☐ Conveyer Oven (Conveyor Belt Oven)
- ☐ Flat Top Griddle
- ☐ Microwave Ovens
- ☐ Steamers
- ☐ Tilt Griddle (or Braiser)
- ☐ Conventional Ovens
- ☐ Deck Ovens (Pizza Deck Oven)
- ☐ Hot Water
- ☐ Steam Kettle
- ☐ Stove with Burners
- ☐ Other _____

Taste Test Instructions

You will be given two menu items. Taste-test one food at a time, in the order presented. Chew and swallow the food normally. Eat three bites of the food, or at least half of the sample. Immediately after eating *each* food, use the rating scales to evaluate your acceptance of the food. Rinse your mouth with water. When you return your tray, the second food item will be given to you. Taste-test the second food item as you did the first.

You will rate how much you *like* or *dislike* each food. You will rate several attributes of each food. Check the number that best describes you feeling about the food. You are not required to eat all of the food. If you do not normally eat this food because you dislike it, please do not evaluate. Your comments for each food are invited and are meaningful.

Important!
Please rate each food *individually*. Do not compare it with the other foods. Please do not talk or comment about the foods to the other participants.

After evaluating all of the foods, please take your tray and evaluation sheet to the attendant.

Please make sure that you **fill each bubble in fully** so that your response may be processed accurately. Check marks, lines, or slashes will not register with the scanning program.

Example:

Incorrect:

Correct:

Date: _____

Taste-Test Subject Questionnaire

Please complete the following questionnaire. Use either an ink pen or a pencil to make dark, solid marks.

The following questions are completed in the bubble grids to the right.
(1) The first 3 letters of your Last Name (i.e. Smith = SMI)
(2) The last 4 digits of your Social Security Number (i.e. 123-45-6789 = 6789)

(2)				
0	O	O	O	O
1	O	O	O	O
2	O	O	O	O
3	O	O	O	O
4	O	O	O	O
5	O	O	O	O
6	O	O	O	O
7	O	O	O	O
8	O	O	O	O
9	O	O	O	O

(1)			
A	O	O	O
B	O	O	O
C	O	O	O
D	O	O	O
E	O	O	O
F	O	O	O
G	O	O	O
H	O	O	O
I	O	O	O
J	O	O	O
K	O	O	O
L	O	O	O
M	O	O	O
N	O	O	O
O	O	O	O
P	O	O	O
Q	O	O	O
R	O	O	O
S	O	O	O
T	O	O	O
U	O	O	O
V	O	O	O
W	O	O	O
X	O	O	O
Y	O	O	O
Z	O	O	O

Personal Information:

Age:
- ⬭ 18 to 21
- ⬭ 22 to 25
- ⬭ 26 to 29
- ⬭ 30 to 35
- ⬭ over 35

Gender:
- ⬭ Female
- ⬭ Male

Ethnicity:
- ⬭ Caucasian
- ⬭ African American
- ⬭ Asian
- ⬭ Hispanic/Mexican American
- ⬭ Native American
- ⬭ Other (please specify) _____

Military Experience:
- ⬭ Yes
- ⬭ No

Daily Information:

Do you have a cold, allergy, nasal, or sinus infection today?

- ⬭ Yes
- ⬭ No

Have you used any tobacco products in the last five hours?

- ⬭ Yes
- ⬭ No

Food Item: Greek Pasta Salad

Fill the circles that best indicate you evaluation of this food. (Mark only one for each category)

How would you rate the **Appearance** of this food?

1	2	3	4	5	6	7	8	9
Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

How would you rate the **Aroma** of this food?

1	2	3	4	5	6	7	8	9
Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

How would you rate the **Taste/Flavor** of this food?

1	2	3	4	5	6	7	8	9
Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

How would you rate the **Texture** of this food?

1	2	3	4	5	6	7	8	9
Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

How would you rate this food **Overall**?

1	2	3	4	5	6	7	8	9
Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Have you ever eaten this food item?

- ⬭ Yes
- ⬭ No

Would you choose this food item again?

1	2	3	4	5	6	7	8	9
⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Extremely Unlikely	Very Unlikely	Moderately Unlikely	Slightly Unlikely	Neither Likely Nor Unlikely	Slightly Likely	Moderately Likely	Very Likely	Extremely Likely

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Comments:

APPENDIX
TASK 7:
STRESS, NUTRITION AND IMMUNE FUNCTION LABORATORY

Figure 1.

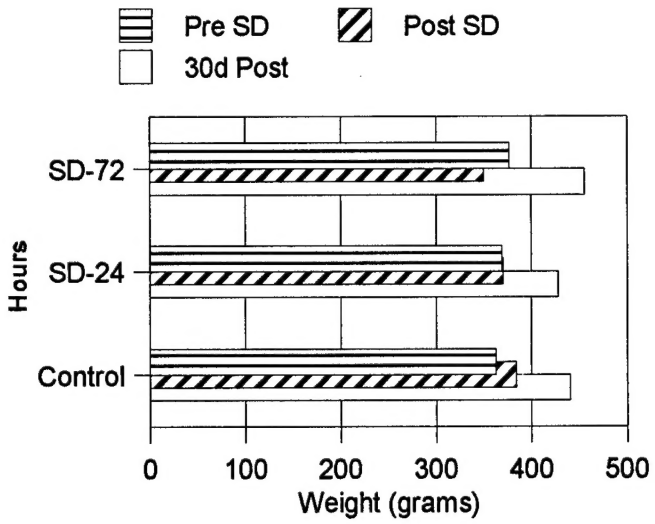

Figure 1. Effect of sleep deprivation (SD) on body weight. Rats were sleep deprived for either 24 (SD-24) or 72 hrs (SD-72) and their body weights determined prior to sleep deprivation (Pre), immediately post sleep deprivation (Post), and at 30 days (30d) later. Control rats were weighed at the same time but not sleep deprived. The results represent the average weights of 4 rats per group.

Figure 2.

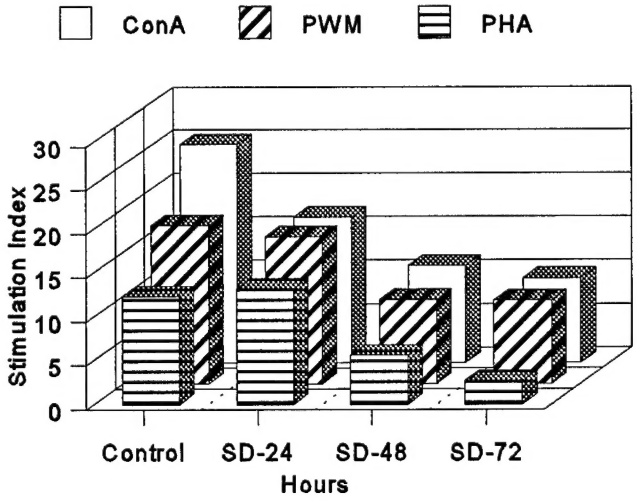

Figure 2. Sleep deprivation suppresses the lymphoproliferative response to mitogens. Rats were subjected to 24, 48 or 72 hours of sleep deprivation. Control rats were housed in the same room but not sleep deprived. Immediately following sleep deprivation, the rats were killed and their spleens removed for *in vitro* stimulation with mitogens. After 72 hours in culture, the cells were pulsed with ^{3}H-thymidine for 4 hours. Labelled DNA was harvested onto glass filter papers for liquid scintillation counting. The results represent the average stimulation index for 4 rats per group.

Figure 3.

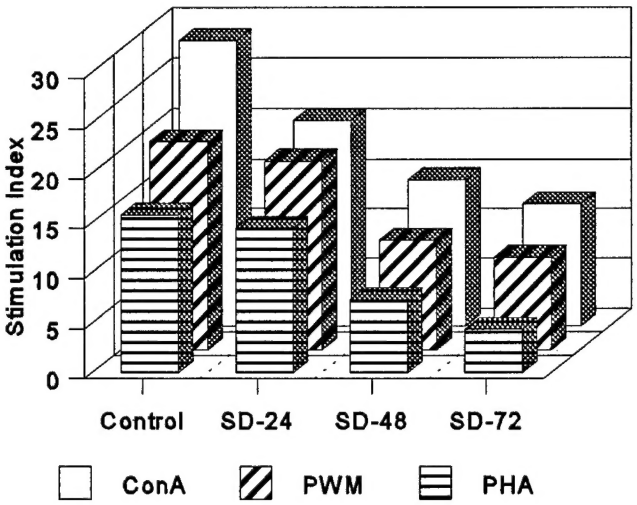

Figure 3. IL-2 fails to overcome sleep deprivation inhibition of the lymphoproliferative response to mitogens. Rats were subjected to 24, 48 or 72 hours of sleep deprivation. Control rats were housed in the same room but not sleep deprived. Immediately following sleep deprivation the rats were killed and their spleens removed for *in vitro* stimulation with mitogens and 60 units/ml of recombinant IL-2. After 72 hours in culture the cells were pulsed with ^{3}H-thymidine for 4 hours. Labelled DNA was harvested onto glass filter papers for liquid scintillation counting. The results represent the average stimulation index for 4 rats per group.

Figure 4.

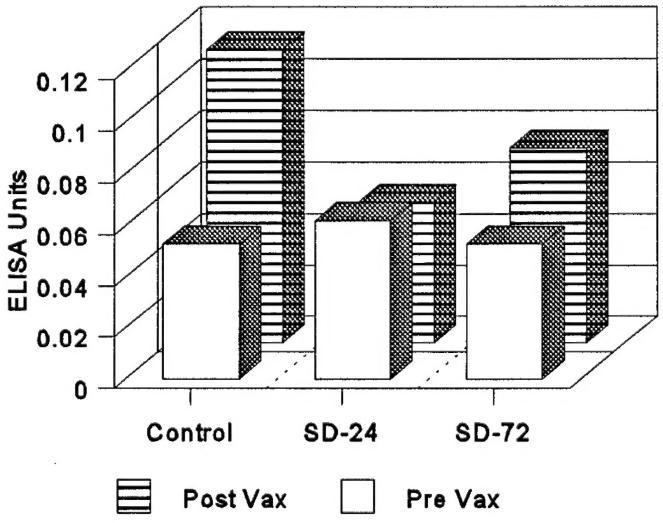

Figure 4. Sleep deprived rats exhibit a reduced antibody response to MBP vaccinaton. Rats were vaccinated immediately prior to sleep deprivation for 24 or 72 hours. Control rats were vaccinated at the same time as the sleep deprived rats. Serum antibody levels to the test antigen, MBP, were determined one month after vaccination by ELISA. Results represent the average of four rats per group.

Figure 5.

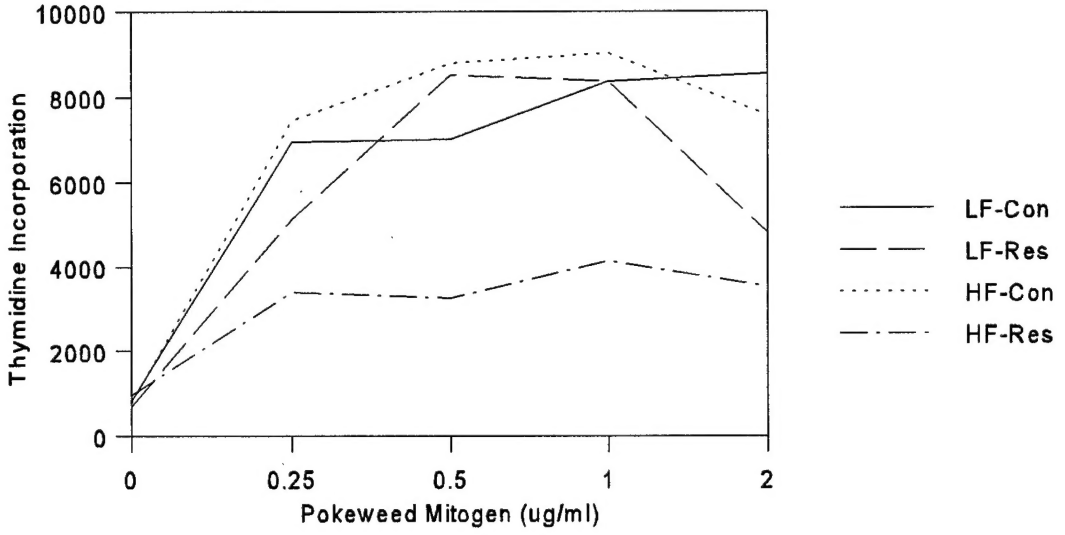

Figure 5. A high fat diet exacerbates restraint stress-induced immune modulation. Rats were placed on either a high fat (HF) or low fat (LF) diet and subjected to four consecutive days of restraint stress (Res). Other rats on the same diets were not restrained and served as controls (Con). Two days after the restraint was completed the rats were killed and their splenocytes were incubated with various doses of pokeweed mitogen for 72 hrs. The cultures were then pulsed with ^{3}H-thymidine for 4 hours and the DNA harvested on glass fiber filters for liquid scintillation counting. The results represent the average of four replicates.

Figure 6

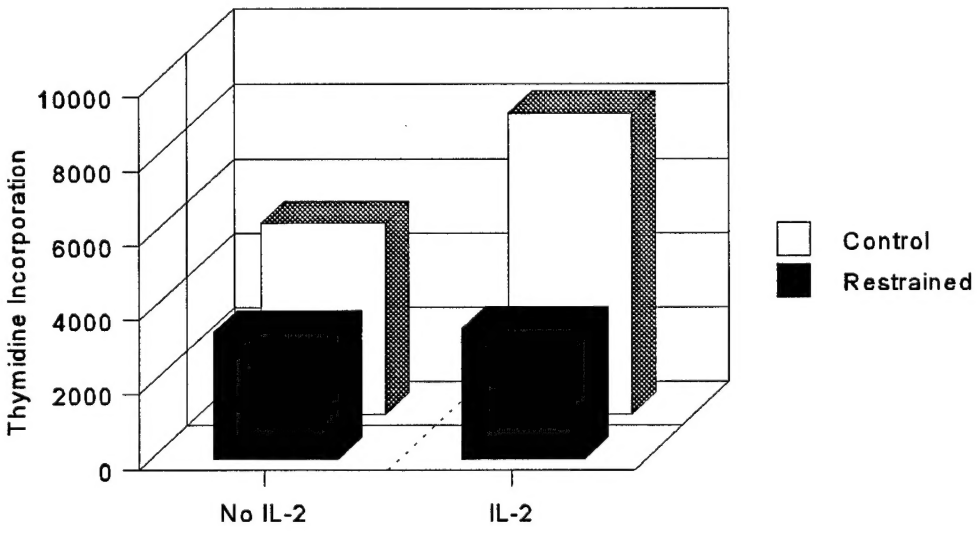

Figure 6. IL-2 fails to overcome the restraint stress-induced inhibition of lymphoproliferation in those rats fed the high fat diet. Splenocyte cultures from restrained or control rats fed a high fat diet were incubated with ConA in the presence or absence of 60 units/ml of recombinant IL-2. The cultures were pulsed with ^{3}H-thymidine and the DNA harvested for liquid scintillation counting. The results represent the average of four replicates.

Figure 7.

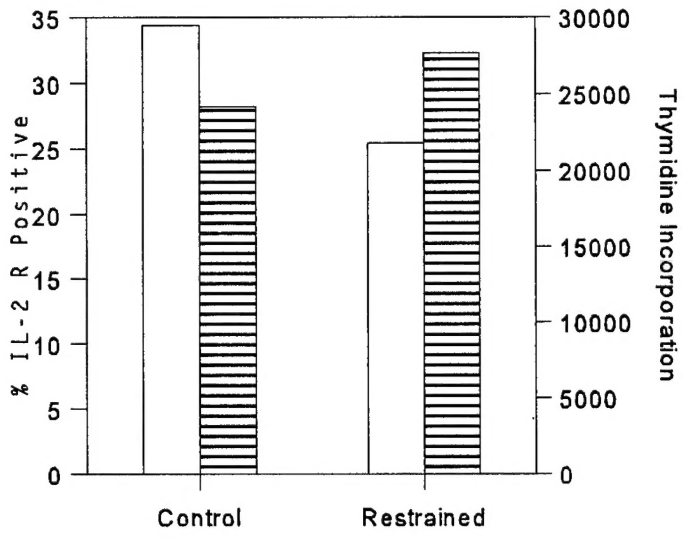

Figure 7. Splenocytes from restrained rats exhibited reduced proliferation and increased IL-2 receptor expression. Splenocytes were obtained from restrained and control rats fed a high fat diet. The cultures were stimulated with PHA for three days and then half of the cells were pulsed with ^{3}H-thymidine (Open Bars) and the remainder stained for IL-2 receptor expression (Striped Bars).